Volume 22 Number 2

JOURNAL of DENTAL RESEARCH

FOUNDED BY WILLIAM J. GIES IN 1919

Official Publication of the

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

April, 1943

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PUBLISHED BI-MONTHLY AT MT. ROYAL AND GUILFORD AVENUES, BALTIMORE, MD.
By THE INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

EDITORIAL OFFICE: 4559 Scott Ave., St. Louis, Mo.

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Subscription price $5.00 per volume. Single numbers when available $1.00.

Entered as second-class matter June 24, 1919, at the Post Office at Baltimore, Maryland, under the act of March 8, 1879

WAVERLY PRESS, INC.

BALTIMORE, U. S. A.

THE PENETRATION OF SILVER NITRATE SOLUTION INTO DENTIN[1]

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For some time past, studies have been carried on with the intention of re-examining commonly accepted beliefs on the use and action of silver nitrate solutions applied to the teeth. It has been found that some existing opinions do not coincide with the facts.

The first part of the present study was reported previously (1). Cavities had been prepared exposing the sound dentin of non-carious teeth. Silver nitrate solutions were applied and precipitated. Decalcified sections from the teeth showed that the silver nitrate product had penetrated the sound dentin. Then carious teeth were treated in the same manner and it was found that the silver nitrate had not penetrated as far as had the bacteria present.

Ireland (2), Richards (3), Woodle (4), and others have reported that the silver nitrate product in carious teeth stopped when it reached sound dentin, stating as a possible explanation that coagulation was produced at the edge of the sound dentin and formed a barrier stopping further penetration. Since our findings were contrary to this theory, the attempt was made to find out what determined the depth of silver nitrate penetration. The two factors varied were: (a) the length of time intervening between the application of silver nitrate solution and the application of the precipitant, and (b) the time intervening between the application of the silver nitrate solution and the removal of the tooth and its fixation for histological study.

Cavities were prepared in dogs' teeth and human teeth. Howe's ammoniacal silver nitrate solution was applied and was precipitated with eugenol. In some cases the length of time between application of the silver nitrate solution and the application of the precipitating eugenol was varied. The intervening periods of time were 1, 3, 5, 10 and 30 minutes, and 24 hours. All teeth were removed and fixed for sectioning 1 week after the application.

In other cases the eugenol was applied 3 minutes after the Howe's silver nitrate solution, but different periods of time elapsed before the extraction and fixation of the teeth. The shortest period of time was about 15 minutes, and the longest was 1 month. The pulps of all teeth were studied to see if the drugs had caused any changes or irritation. The histological technique[3] was such as to avoid disturbance of the silver nitrate products in the tooth.

[1] Received for publication Feb. 8, 1943.

[2] Now at Tufts College Dental School.

[3] Using a combined fixing and decalcifying solution consisting of: 50 grams trichloracetic acid; 150 cc 40% formaldehyde make up to 500 cc with water.

The accompanying figures, photomicrographs of sections from human teeth, show the results obtained in both human and dog teeth. The teeth shown in the first 6 figures were left in the mouth 1 week after silver nitrate application.

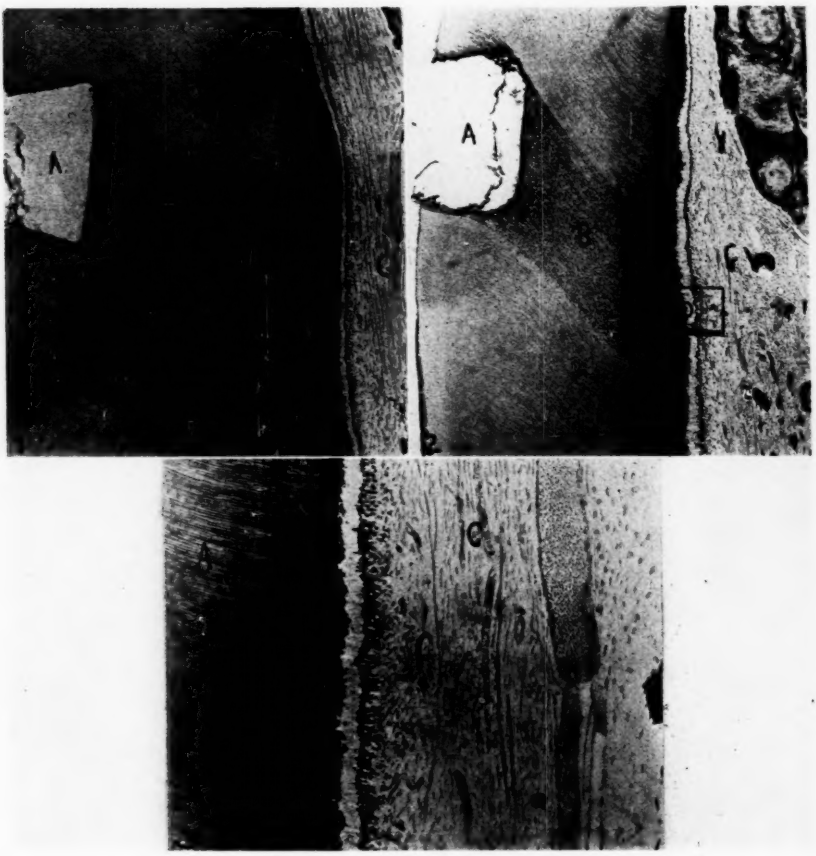

FIG. 1. Silver nitrate application for 1 minute
A—Cavity, B—Silver Nitrate Precipitate, C—Pulp
FIG. 2. Application of silver nitrate for approximately 24 hours
A—Cavity, B—Silver Nitrate Precipitate, C—Pulp
FIG. 3. Higher magnification of pulp in fig. 1 opposite cavity
A—Dentin, B—Odontoblasts, C—Pulp

Fig. 1 is a photograph of a section from a tooth of a patient 56 years of age. The cavity was cut in the gingival third of the labial surface of the upper right canine. Silver nitrate was applied and left in place for 1 minute, the cavity was dried and eugenol applied and left for 5 minutes. The deepest point of penetration of the silver nitrate can be seen at B. It has passed into the dentinal tubules to a depth of 1.2 mm. and is 1 mm. from the pulp. The pulp appears

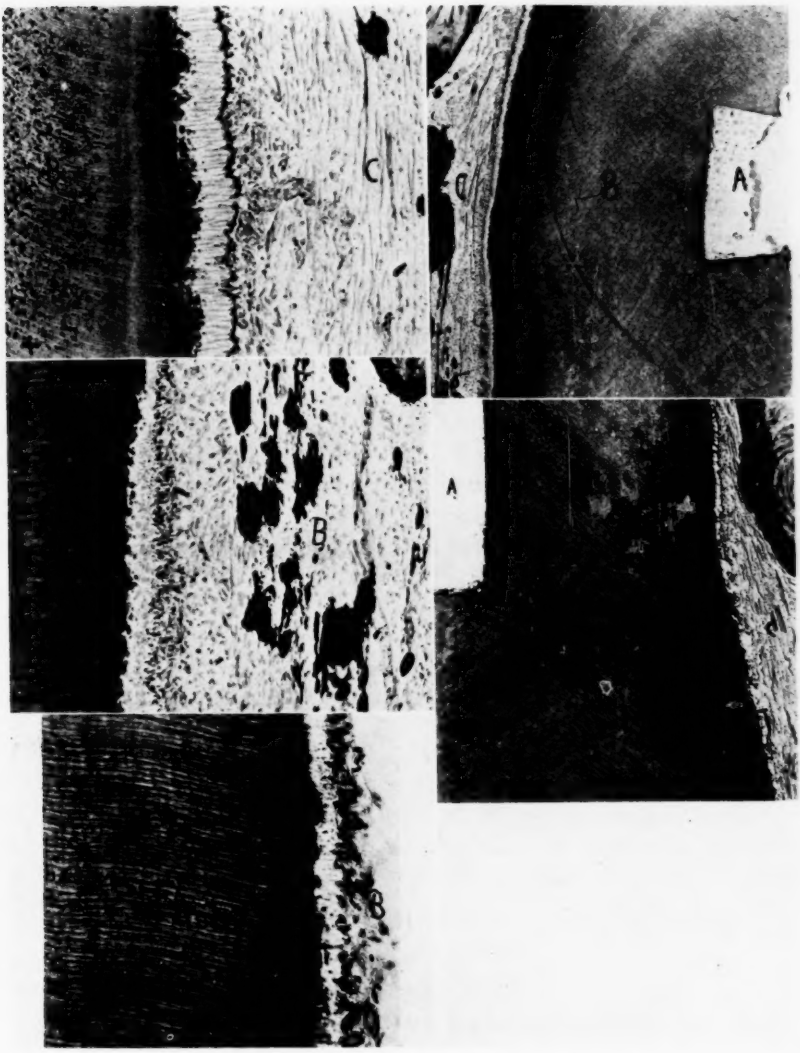

FIG. 4. Higher magnification of area D in fig. 2
B—Silver Nitrate Precipitate, C—Pulp
FIG. 5. Control tooth—no silver nitrate application
A—Cavity, B—Dentin, C—Pulp
FIG. 6. Higher magnification of pulp area in fig. 5 opposite cavity (reversed)
A—Dentin, B—Pulp
FIG. 7. One month after $AgNO_3$ application
A—Cavity, B—Silver Nitrate Precipitate, C—Pulp
FIG. 8. Higher magnification of area D in fig. 7
A—Silver Nitrate Precipitate, B—Pulp

normal, though it may be abnormally vascular for a patient of this age. *Fig. 3* shows area C from *fig. 1* under higher magnification. *Fig. 2* is a photomicrograph of the upper left second incisor of the same individual. The cavity A, was cut to approximately the same depth as in all other teeth. The silver nitrate was left for 24 hours before it was precipitated by eugenol. By the time eugenol was applied some of the silver nitrate had been already precipitated. The silver nitrate has penetrated 1.65 mm. deep into the dentin, and is 0.18 mm. away from the pulp. The only reaction of the pulp is a slight hemorrhage opposite the cavity. *Fig. 4* is a higher magnification of area D in *fig. 2*; B, indicates the silver nitrate, C, blood filled capillaries.

Fig. 5, is a photomicrograph of the upper left canine of the same individual as in *figs. 1* to *4*. This tooth was used as control. No silver nitrate was applied to the cavity. There is some disturbance of the pulp in and near the odontoblastic layer opposite the cavity. This disturbance is entirely due to the cavity preparation, and is of the same type and the same severity as in the specimens treated with silver nitrate. Apparently, the cavity preparation and not the silver nitrate caused the pulp changes. The changes are so slight that it may be assumed that the pulps will be able to recuperate. *Fig. 6* is a higher magnification of area C, in *fig. 5*, showing the disturbance in the odontoblastic layer, and calcification in the pulp tissue.

Figs. 7 and *8* are photomicrographs of sections of a tooth which was extracted 1 month after the silver nitrate application. The precipitate reached the pulp, where some reaction occurred. In the teeth extracted shortly after the application, and in those left in the mouth for a week, the silver nitrate had not reached the pulp.

To determine the effect of repeated applications of silver nitrate solution, 3 cavities were cut in a line along the buccal surface of a dog's lower canine tooth. Silver nitrate solution was applied and precipitated twice in the upper and lower cavities, and once in the middle cavity. There was no appreciably difference in the depth of penetration.

COMMENTS

Silver nitrate has been widely used in the belief that it is self limiting in its power to penetrate dentin. The explanation was that in sound dentin each tubule contains a protein fibril which is precipitated by the silver nitrate, forming a barrier to further penetration. From this it was thought that the penetration would extend only to the sound dentin. This theory does not fit the present facts, since not only is sound dentin penetrated, but the depth of penetration increases as time goes on. So far as the present measurements show, the length of time intervening between the application of silver nitrate and the application of the precipitant makes no difference in the depth of penetration. Such slight differences as may have been present immediately after precipitation may have been masked by the greater depth of penetration permitted by the week intervening before extraction and fixation of the teeth. However, since silver nitrate is applied with the intention of stopping decay and saving the tooth, the small

differences of penetration that may be obtained by different techniques are immaterial, because before long the silver nitrate product will have penetrated all the way through the dentin in any event.

It is noted that the damage to the pulp was so slight that it may probably be disregarded and not considered an argument against the use of silver nitrate. This is in agreement with common experience. Reports of pulp disturbance following the use of silver nitrate are rare.

The mechanism of the continued penetration by the silver nitrate or its product is not explained by the present findings. It seems likely that the precipitation of the Howe's solution in the dentin produces a finely divided or colloidal silver or silver proteinate which might be carried by the dental lymph.

CONCLUSIONS

Several conclusions may be drawn from these experiments:

1. Howe's silver nitrate solution penetrates sound dentin.
2. The length of time of application of the silver nitrate solution before its precipitation, does not appreciably influence its depth of penetration.
3. The depth of penetration increases as time goes on after the application and precipitation, the products eventually reaching the pulp.
4. Penetration of silver nitrate or its product through the sound dentin does not irritate the pulp severely.
5. Repeated applications of silver nitrate did not appreciably change the depth of penetration.

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THE EXISTENCE OF CUTICULAR STRUCTURES ON HUMAN TEETH[1]

IRVING GLICKMAN AND BASIL G. BIBBY

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An external covering over the completed enamel surface is described in the literature as a cuticular formation (1), an amorphous pellicle (2), a hornlike substance (3), or a combination of an organic membrane which may be calcified and keratinous cuticle (4). This covering is presented as consisting of a single layer (3), and as consisting of two layers (1, 5, 6). Its origin is attributed to the ameloblasts (3), the entire enamel organ (1, 5), and a combination of ameloblasts and epithelial attachment (6). It has been termed "Nasmyth's membrane", "enamel cuticle" (3), or "primary" and "secondary enamel cuticle". An additional cuticle which is frequently found covering the cementum has been described as a continuation of the secondary enamel cuticle and a derivation of the epithelial attachment. It has been termed the "dental cuticle" (6).

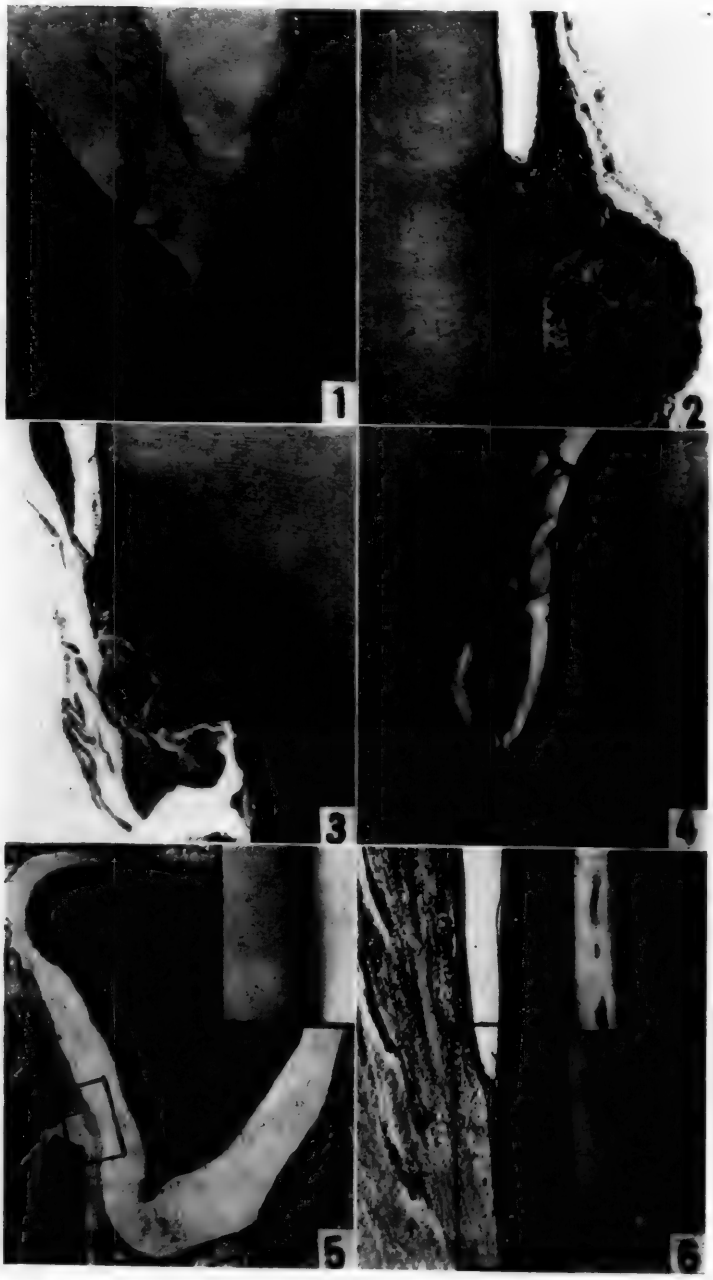
A study of approximately 1400 decalcified sections of 64 erupted teeth in situ in human adult jaws offered an opportunity to establish more definitely whether or not cuticular structures actually exist in relation to the teeth and periodontal tissues. Three unerupted teeth completely embedded in the maxilla were included in the series studied. In general, the histological technique used in the preparation of the material was that presented in a previous publication (7). The term cuticle when used here refers to a thin, strandlike, non-cellular structure with a clear eosin-staining matrix of varying widths enclosed within smooth linear margins.

ENAMEL SURFACES

In decalcified sections a strand-like eosin staining structure approximately 1.5 micra in width with smooth linear margins was seen at the periphery of the space previously occupied by the enamel which had been removed by the decalcifying agent. In erupted teeth this cuticle persisted at the enamel surface adjacent to the cemento-enamel junction in almost all cases (*figs. 1, 2, 3*) and was observed less frequently at the bottom of developmental grooves on the occlusal surface of posterior teeth (*fig. 4*).

In the unerupted teeth, completely embedded in the maxilla, a similar cuticle was observed subjacent to the connective tissue capsule which enveloped the crown (*fig. 5*). This finding indicates that an acid resistant cuticle, in no way of itself derived from the oral epithelium in the course of tooth eruption, covers the completed enamel before the tooth erupts into the oral cavity. However, that it is the only source from which the cuticular structure which covers the enamel

[1] Received for publication November 30, 1942.



FIGS. 1–6

of the erupted tooth originates cannot be positively stated. This is particularly so in view of the fact that our observations on the cementum make it appear likely that a cuticular element may be added to the external surface of the tooth as it erupts into the oral cavity. Nevertheless, since the cuticle present before eruption is the constant source of cuticular covering for the enamel of the erupted tooth and since it is limited only to the enamel we feel that it is logical, even though we recognize the possibility of adventitious elements from other sources, to use the term "enamel cuticle" to describe the cuticular structure as we observed it on the enamel surface of the erupted tooth.

CEMENTUM SURFACES

In approximately one half of the sections studied, a thin, non-cellular strand of eosin staining material enclosed within smooth linear margins was observed in close apposition to cementum surfaces (*figs. 6, 7*). Its average width was approximately 2.3 micra. It occurred only on the cementum surface which had been traversed by the receding epithelium of the gingival crevice after the latter had progressed in an apical direction, and was not found on the cementum surfaces which were covered by periodontal membrane.

The morphology of the epithelium of the gingival crevice in the region of attachment indicated a possible formative relationship between it and the cuticle. The epithelium was generally multilayered and presented a large number of cells in close apposition with the tooth structure. Cytoplasmic processes of the epithelial cells appeared to effect the attachment of tooth to epithelium in some instances (*fig. 8*). The cuticle originated in the area of attachment (*fig. 6*) and could often be traced for the entire length of the exposed cementum. Cellular alterations in the epithelium in the region of attachment to the tooth point to the origin of this cuticle. The nuclei were absent and the cells fused to form a con-

FIG. 1. Cemento-enamel junction. Cuticular remnant at periphery of space occupied by enamel. Hematoxylin & eosin. Orig. mag. ×167

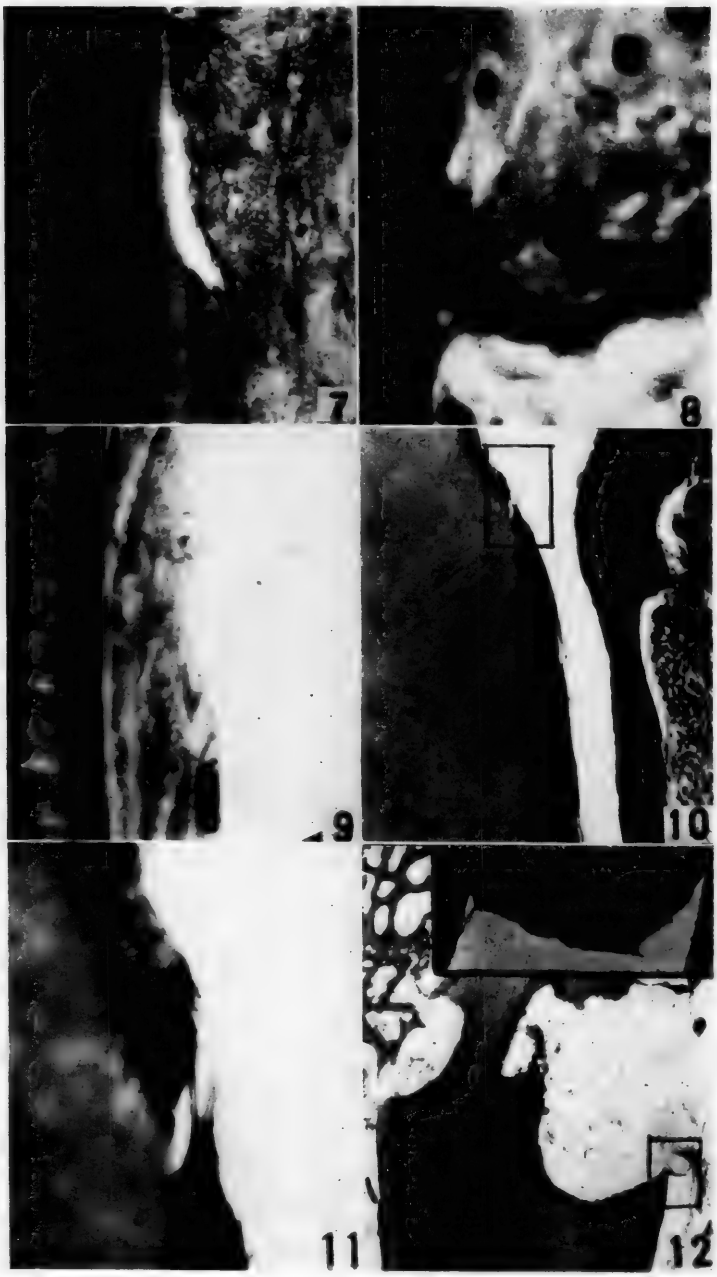
FIG. 2. Cemento-enamel junction in patient with periodontoclasia. Indentation in cementum separated by enamel cuticle from space previously occupied by enamel. Hematoxylin & eosin. Orig. mag. ×60

FIG. 3. Cemento-enamel junction. Pronounced cavitation in dentin which extends in pulpal direction and undermines area previously occupied by enamel. There is no break in the continuity of the cuticle at the periphery of the enamel. Hematoxylin & eosin. Orig. mag. ×60

FIG. 4. Developmental groove on occlusal surface of molar showing remnants of enamel and enamel cuticle. Hematoxylin & eosin. Orig. mag. ×660

FIG. 5. Labio-palatal section through crown of unerupted tooth completely embedded in maxilla. Hematoxylin & eosin. Orig. mag. ×8 Insert *fig. 5*. Cuticle with smooth linear margins at periphery of space previously occupied by enamel immediately subjacent to enveloping connective tissue capsule. Hematoxylin & eosin. Orig. mag. ×2200

FIG. 6. Gingival crevice at cementum in patient with periodontoclasia. Cuticular strand extends in coronal direction along cementum from region where epithelial cells are attached to tooth. Hematoxylin & eosin. Orig. mag. ×166 Insert *fig. 6*. Transposed crevicular cuticle. Origin of clear staining linear cuticle in apposition with cementum in region where epithelium is attached to tooth. Hematoxylin & eosin. Origl mag. ×340



FIGS. 7–12

tinuous cuticle. The cytoplasm appeared as a continuous amorphous mass which assumed the hyalin-like clear staining quality of the formed cuticle (*fig. 9*). Unaltered epithelial cells stained blue with hematoxylin-eosin; the formed cuticle appeared pink.

The term "transposed-crevicular cuticle" is suggested for this structure because it indicates a change in relationship of a cuticle which is the result of cytomorphic changes in the epithelial cells of the gingival crevice. Since the gingival crevice traverses the enamel in the course of tooth eruption it is logical to assume that the cuticle formed from the epithelium of the gingival crevice is likely to be superimposed upon the cuticular covering of the enamel which had been formed previous to tooth eruption (*figs. 10, 11*). In such instances, the designation of the structure formed previous to eruption, as the enamel cuticle, and the superimposed structure deposited in the course of eruption, as the transposed-crevicular cuticle, properly infers the derivation of each and avoids the ambiguity which the terms "primary" and "secondary enamel" cuticle have created, particularly in so far as the relationship to cementum is concerned.

RELATION OF ENAMEL CUTICLE AND TRANSPOSED-CREVICULAR CUTICLE TO TOOTH DESTRUCTION AND PERIODONTOCLASIA

Observations made of the enamel cuticle and transposed-crevicular cuticle at the cemento-enamel junction in patients with periodontoclasia revealed that while caries-like destruction originating at the cementum surface progressed apically and pulpally, contiguous enamel surfaces were not involved (*figs. 2, 3*). There is a possibility, therefore, that the destruction was prevented from affecting the external enamel surface by the presence of the enamel cuticle. In those areas where an intact transposed-crevicular cuticle covered the cementum, there was

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FIG. 7. Cementum denuded by recession in patient with periodontoclasia. Clear staining cuticle in apposition with cementum surface separates it from calculus. Hematoxylin & eosin. Orig. mag. ×600

FIG. 8. Epithelial cells in region of attachment to cementum present vacuolization of cytoplasm. Cytoplasmic strands of epithelial cells continuous with cementum surface. Hematoxylin & eosin. Orig. mag. ×700

FIG. 9. Remnant of epithelium in region of attachment to tooth showing absence of nuclei and fusion of cells to form a continuous cuticle in apposition with cementum. Hematoxylin & eosin. Orig. mag. ×580

FIG. 10. Cemento-enamel junction in patient with periodontoclasia. Strand-like structure extends from cementum surface on to remnant of enamel. Hematoxylin & eosin. Orig. mag. ×126

FIG. 11. Detail of *fig. 10*. Two cuticles at the periphery of the space previously occupied by the enamel. The enamel cuticle is on the inner aspect of the cementum and the transposed crevicular cuticle, which is external to the enamel cuticle, extends in an apical direction along the outer cementum surface. Hematoxylin & eosin. Orig. mag. ×540

FIG. 12. Unerupted canine embedded in maxilla. Space filled with connective tissue and lymphocytes extends from exterior of crown to pulp cavity on one aspect and partially into dentin on other. Hematoxylin & eosin. Orig. mag. ×7. Insert *fig. 12*. Continuity of enamel cuticle is broken. Connective tissue and lymphocytes fill space previously occupied by enamel. Hematoxylin & eosin. Orig. mag. ×60

no destruction. However, because destroyed cementum was found while adjacent enamel surfaces remained intact, it seems that the surface protective action of the transposed-crevicular cuticle may be less effective than that of the enamel cuticle. It is possible that the greater protective action of the enamel cuticle resulted from its combination with an added overlying transposed crevicular cuticle. Microscopic observations, however, revealed only a single cuticle in the enamel area in these cases.

In an unerupted tooth, completely embedded in the maxilla, irregularly concave areas were seen in which the enamel and dentin had apparently been destroyed and replaced by connective tissue cells and lymphocytes (*fig. 12*). While the mechanism responsible for the destruction of embedded tooth structure remains to be clarified, it is known that the destruction observed here originated at the periphery of the enamel and progressed in a pulpal direction. The point of interest here is the fact that although the enamel cuticle is acid resistant, it had been destroyed previous to the involvement of the underlying tooth structure. This seems to indicate that the forces of destruction in the unerupted tooth are different from those occurring in the erupted tooth.

SUMMARY AND CONCLUSIONS

Two acid-resistant cuticles are described in relation to the erupted tooth and periodontal tissues. The term "enamel cuticle" is suggested for the cuticle which is the constant finding on the enamel only in both erupted and unerupted teeth. The term "transposed-crevicular cuticle" is suggested for the cuticle which is derived from the epithelium of the gingival crevice and is transposed upon the tooth surface in the course of eruption. It was observed in approximately 50 per cent of the cases studied. The latter cuticle is most commonly seen on the cementum but may also exist on the crown external to the enamel cuticle.

It is suggested when the cementum is exposed by periodontoclasia and secondarily involved by caries, the enamel cuticle possesses a greater surface protective action than does the transposed-crevicular cuticle.

The authors are indebted to Anna Morse, M.S., Assistant in Oral Pathology, for the preparation of the microscopic material and to Dr. H. Spencer Glidden, Pathologist, Tewksbury State Hospital and Instructor in Pathology and Bacteriology, Tufts College Medical and Dental Schools, for making a large portion of the autopsy material available for study.

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A FILAMENTOUS MICROORGANISM ISOLATED FROM STAINED TEETH[1]

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A bacteriological study was made of a brown deposit firmly adherent to the labial surfaces of a man's anterior teeth. Films prepared from portions of the deposit, scraped from the teeth by means of a sterile scaler, were stained by Gram's method. Many Gram-positive filaments were observed together with some cocci. Some of the deposit suspended in sterile salt solution was streaked on rabbit's blood agar plates and incubated at 37°C., under aerobic and anaerobic conditions. After 3 days incubation the plates were examined. The aerobic plates contained colonies of a *Staphylococcus aureus*, a Gram-negative coccus and a green producing streptococcus, the latter the predominant organism. The anerobic plates in addition to the coccus colonies had a moderate number of minute greyish white aborescent colonies tenaciously adherent to the surface of the medium. These colonies consisted of Gram-positive filaments. Attached to the ends of some of the filaments were thick bacillary bodies. The filamentous organism after several subcultures on blood agar under anaerobic conditions became adapted to its environment and grew vigorously in 48 hours. Transfers of these subcultures at first would not grow under aerobic conditions, but later ones developed colonies which were only half the size of those grown under anaerobic conditions. Continued aerobic subcultivation, however, resulted in good growth. A study of the morphological and cultural characteristics of this filamentous microorganism together with a discussion of its classification follows.

CHARACTERISTICS OF THE MICROORGANISM

Infusion agar with 10 per cent whole autoclaved saliva. Greyish white aborescent colonies 1 to 2 mm. in diameter developed after 48 hours incubation. One week old colonies assumed a more umbonate character. They were very tenacious and removed with difficulty from the surface of the medium (*fig. 1*). The colonies consisted of Gram-positive filaments, which throughout their length often contained granules of varying size. The filaments were curved, frequently irregular and of varied length. Attached to the ends of some filaments were thick bacillary bodies either square or oval in shape (*fig. 2*). Colonies several weeks old possessed filaments of varied thickness which contained larger granules. Evidence of branching was noted rather infrequently.

Ascitic fluid infusion agar. Growth was similar to above. Films of the

[1] Presented at a meeting of the New York Section of the International Association for Dental Research, March 1941. Received for publication November 27, 1942.

colonies stained by Gram's method, Ziehl Neelsen's method or with the Giemsa stain all showed what appeared to be a sheath or capsule about the filaments (*fig. 3*). Henrici (9) suggests this capsular appearance to be an artefact resulting from shrinkage of the medium away from the organism during the drying of the film. His opinion appears to be correct since this condition was not observed with any other medium employed and could not be duplicated on numerous attempts employing other lots of the same medium.

Glucose infusion broth. Small individual granules developed at the bottom and sides of the tube, some of which adhered to the walls of the tube. The supernatant broth remained clear during 1 month's incubation. Shaking of the tube caused the granules to ascend into the clear broth but did not cause their rupture. Eventually they were again deposited at the bottom of the tube.

Infusion gelatin. No liquefaction during a period of 1 month's incubation. The microorganism grew as small granules throughout the gelatin.

Litmus milk. No change occurred in the milk during a period of 6 week's incubation.

Potato slants. When first subcultured the microorganism would not develop on the potato slants. Successful growth was obtained later after cultures transferred for several months were employed as inoculum. Growth was greyish white, scanty and had a dry appearance.

Carbohydrates. (Sugar free infusion agar base with 1 per cent of various carbohydrates was employed.) Fermentation with acid but no gas occurred in dextrose, maltose, saccharose, dextrin and starch. No fermentation was indicated with mannite, lactose, xylose, raffinose, rhamnose, dulcitol or glycerine. The tests were observed during a 1 month period of incubation.

Animal inoculations. Guinea pigs and mice were injected either subcutaneously or intraperitoneally with heavy suspensions of the microorganism in salt solution. No apparent symptoms developed. Animals were autopsied after 2 week and 2 month periods respectively. No gross lesions were noted.

Growth on infusion agar was poor, on serum infusion agar it was very scanty and no growth was obtained on Saboraud's agar, extract agar, corn meal agar, bread agar and peptone water.

DISSOCIATION OF THE MICROORGANISM

The filamentous microorganism was grown aerobically for 5 days at 37°C. on 2% soluble starch infusion agar (*figs. 4* and *5*). Dissociation of the rough dry tenacious form of colony into a moist smooth type occurred which was easily removed from the surface of the medium. The smooth colony consisted mainly of bacillary forms of a diphtheroid character together with a few filaments (*fig. 6*). The smooth form possessed the same fermentative characteristics as the rough tenacious colony. Litmus milk and gelatin were not changed. It was not pathogenic for animals. In contrast to the rough tenacious type of colony, the smooth form grew readily on potato slants, producing slimy, light brownish pigmented growth. This type of growth consisted of irregular coccoid elements with very few bacillary or filamentous forms. The slimy growth from the potato when

inoculated on the surface of saliva infusion agar plates reverted to the smooth colony type containing mostly the bacillary diphtheroid form. Further reversion to the dry tenacious type has not been obtained.

DISCUSSION

The characteristics of the filamentous microorganism would seem to warrant its inclusion in the genus Actinomyces (*fig.* 7). Much confusion persists, however, concerning the properties of microorganisms designated as Leptotrichia and Actinomyces. Bergey's (1) *Manual of Determinative Bacteriology* includes both of these genera in the family Actinomycetaceae with the chief difference between them noted as the occurrence of true branching among the Actinomyces. The manual's description of the genus Leptotrichia is as follows:

> "Thick long straight or curved filaments, unbranched, frequently clubbed at one end and tapering at the other. Gram positive when young. Filaments fragment into short rods. Anaerobic and facultative. No aerial hyphae or conidia. Parasites or facultative. Typical species, *Leptotrichia buccalis*, (Robin) Trevisan."

Listed as species of this genus are the *L. buccalis* and the *L. placoides*. It would seem that this latter microorganism should be classified as an Actinomyces since it possesses according to the manual's own description, "branching filaments resembling those among the actinomyces."

Thjøtta, Hartman and Bøe, (2) took issue with the *Manual of Determinative Bacteriology* concerning the grouping of Leptotrichia with Actinomyces. They stated (page 36),

> "It is very unlikely that this microbe has anything in common with the Actinomyces, with whom it is classed by Bergey, being one of the genera under the family Actinomycetales. It will not, namely, under any condition, show any sign of branching, and does not even show the typical mode of division of the Cornybacterium. It seems more likely to us that this microbe should be properly classed somewhere among the Bacteriaceae."

Thjøtta and his associates suggested the elimination of the old individual names given so-called species of Leptotrichia. They suggested that only one species, the *Leptotrichia buccalis* under the genus Leptotrichia Trevisan, be recognized until further definite investigation indicates the occurrence of other species. Thjøtta, Hartman and Bøe stated the chief characteristics of the Leptotrichia Trevisan as: an unbranching long rod, Gram-positive when young, without spores but often forming granules in the bacterial body. It never ferments mannitol or liquefies gelatin. The filaments only divide in segments, but never in coccoid bodies.

Grythe (3) concluded from his investigations of oral leptotrichia, that these microorganisms and the actinomyces possess a certain number of similar characteristics such as (a) occurrence as thread, short rod or coccoid forms (b) fragmentation of filaments (c) ability to exist as facultative anaerobes. Grythe asserted, however, that only the actinomyces show true branching.

Nieber (4) studied 11 strains of filamentous microorganisms isolated from the oral cavity. He compared them with strains of 2 true actinomyces and 4 of Gins' leptotrichia. Nieber considered 9 of his 11 strains to be actinomyces since

at some stage during his study true branching was observed. The remaining 2 non-branching strains he classified as leptotrichia. He also mentioned that Gins identified true branching filamentous bacteria as actinomyces.

The confusion which exists as to what filamentous bacteria should be classified as Leptotrichia is further exemplified by the observations of Bibby and his associates. Bibby and Berry (5) reported a study of 83 strains of filamentous bacteria obtained from the oral cavity. They cassified their strains tentatively into 7 groups, stating (page 272):

"Since it is only in Group I that the constituent strains show uniform cultural and biochemical properties, the suggestion of a specific name is warranted for this single group only. For these organisms, we propose the name Leptotrichia buccalis, making this recommendation in full cognizance of Bergey's use of the name for another species. This suggestion is made because, as had been pointed out elsewhere (Bibby, 1935), we consider the organisms of the species in question, Leptotrichia buccalis (Robin) Trevisan, to be bacilli which have no place in the genus Leptotrichia."

The characteristics of Bibby and Berry's group I strains are similar to those of the filamentous microorganism isolated from the stained deposit adherent to the man's teeth. Bibby and Berry observed 3 types of colonies (page 265):

"Short round forms were most prominent in smooth colonies; branched, forked, swollen, and irregular elements in intermediate colonies; and filamentous square-ended forms in rhizoid colonies."

Bibby and Knighton (6) stressed the confusion existing in the classification of actinomyces and other filamentous bacteria. They question whether some of the types reported by Naeslund as actinomyces are not really leptotrichia, stating

"Since the morphologically similar Leptotrichia (Bibby and Berry) give similar appearances of branching in colonies, examined at the magnification used by Naeslund (260 ×) we feel he has not submitted sufficient evidence to justify accepting his organisms as Actinomyces."

From the preceding review of recent literature concerning filamentous bacteria it is evident that the views of investigators are varied concerning the characteristics of microorganisms classified as Leptotrichia. With the exception of Bibby, Berry and Knighton (5, 6), branching is not considered a trait of Leptotrichia.

Bibby (7), from examination of photomicrographs of the microorganism isolated from the dental stain, stated to the writer that he had not observed the same degree of branching in any of his Group I strains.

A description of the microorganism and the photomicrographs of *figs. 1* to *6* (*fig. 7* was not obtained until several months later) were forwarded to Dr. S. A. Waksman (8) for his opinion. He replied:

"Most of the characteristics of this organism appear to fit well with the group of Leptotrichia Trevisan. . . . However there are several disturbing features about your description, namely, the appearance in one or two of the figures of definite branching, which would seem to indicate possible relationship to the Proactinomyces or Cohnistreptothrix type of growth. The fact, however, that this organism produces definitely a sheath, (a phenomenon not

observed among the actinomyces) may possibly indicate the branching is not true but of a false type, produced by the appearance of a sheath."

At the suggestion of Dr. Waksman the description and photographs (except *fig. 7*) were forwarded to Dr. A. T. Henrici who expressed the following opinion:

"I am afraid that I cannot tell precisely what your organism is but certainly I would consider it an Actinomyces (in the broad sense) rather than a Leptotrichia. The sheath is

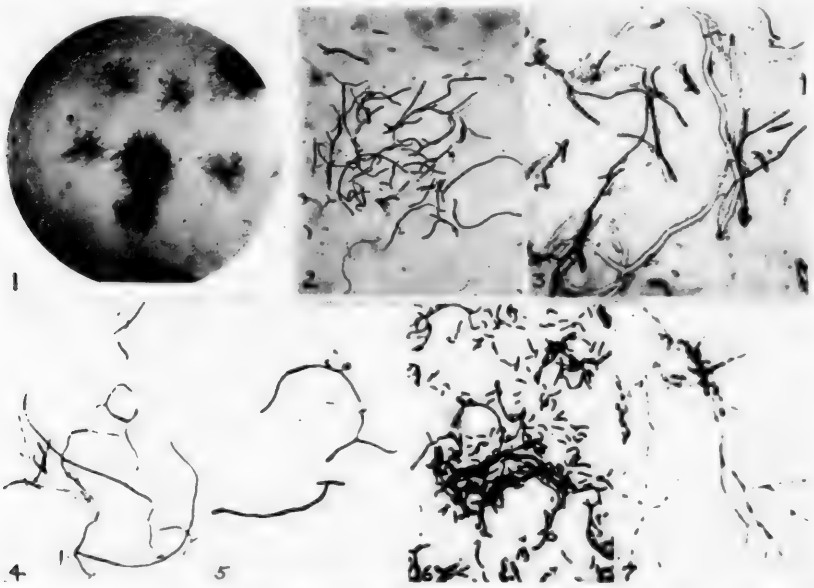

FIG. 1. Aborescent colonies, 48 hours incubation, saliva infusion agar. (Orig. mag., 20 ×)

FIG. 2. Filaments irregular, curved, some with clubbed ends, 3 days incubation, saliva infusion agar. (Orig. mag., 800 ×)

FIG. 3. Sheath-like structure surrounding filaments (may be artifact, see text), 3 days incubation, ascitic fluid dextrose agar. (Orig. mag., 1000 ×)

FIG. 4. Irregular branching of filaments arising from bacillary bodies, 2 per cent starch infusion agar, 4 days incubation. (Orig. mag., 800 ×)

FIG. 5. Individual filaments germinating from bacillary bodies, 4 days incubation, starch infusion agar. (Orig. mag., 1200 ×)

FIG. 6. Bacillary diphtheroid forms, smooth colony type obtained by dissociation of rough tenacious colony. 4 days incubation, starch infusion agar. (Orig. mag., 1200 ×)

FIG. 7. Side branching of filaments, 7 days incubation, saliva infusion agar. (Orig. mag., 1000 ×)

very interesting. You have not said anything about the method of preparing slides, and I am wondering if perhaps these slides were made from a serum medium. Often when such smears are dried, the serum retracts from the organism and gives the appearance of a sheath which is however an artifact. The swollen ends of the filaments occur quite frequently in cultures of Cohnistreptothrix."

Dr. Henrici also mentioned the similarity of this organism with an actinomyces strain isolated by Slack (10) from pockets in areas of periodontosis, and which

injected into rabbits produced actinomycotic lesions. Slack's strain, however, fermented lactose and mannite while the writer's strain did not.

The sheath occurred in films prepared from a 24 hour blood agar slant which was a transfer of a week old growth on glucose ascitic fluid agar. I have tried to duplicate the appearance of the sheath, but have failed. The use of various media, drying the films rapidly even with the aid of heat have not been successful. Therefore since the sheath was only observed on one medium and could not be reproduced, I am inclined to agree with Dr. Henrici that it was an artifact.

SUMMARY

A filamentous microorganism isolated from dental stain on a man's anterior teeth was studied. The literature pertaining to the genera Leptotrichia and Actinomyces is very confused since the opinions of investigators vary as to the characteristics of the microorganisms so classified. The identification of a filamentous organism is thus rendered difficult. The filamentous microorganism in question possessed properties peculiar to both genera. Its identification, however, as an actinomyces would seem to be indicated.

The author wishes to thank Dr. Rhoda Benham, Mycologist, Department of Dermatology, and Dr. Theodore Rosebury, Department of Bacteriology, School of Medicine, Columbia University, for their suggestions and aid in this study.

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INCIDENCE OF UREASE PRODUCING BACTERIA IN SALIVA[1]

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The use of carbamide (synthetic urea) in a mouth rinse has been suggested because in the mouth the carbamide is converted into ammonium carbonate which acts as an efficient buffer for acids. The combination of carbamide and quinine has been recommended as an antiseptic for oral use; the alkalinity resulting from the conversion of carbamide makes the quinine much more germicidal (2). Other therapeutic properties of carbamide such as ability to increase capillary proliferation, stimulate tissue granulations and dissolve necrotic tissue are probably of minor importance in the use of carbamide as an oral rinse.

Carbamide is converted into ammonium carbonate by heat or by the action of an enzyme called urease, which is found in saliva in minute quantities and also is produced by many species of proteolytic bacteria. No investigations of the urease-producing bacteria of the mouth could be found in the literature, so this study was undertaken to determine their incidence in the oral flora.

Shulte and Thompson (3) studied the urea-splitting bacteria in urine and found potent urea-splitting organisms as part of the normal urethral flora in fifteen of two hundred cases. They also found such bacteria associated with renal calculae of the calcium carbonate and calcium phosphate type, and believed the presence of these bacteria predisposed to severe infection. To prevent and treat such infections, these men used a weak lactic acid solution (0.25 to .5%). *Proteus ammoniae*, *Proteus morganie*, *Micrococcus ureae*, certain strains of *Staphylococcus aureus* and certain strains of diphtheroids were found to be of clinical significance. The method used by Shulte and Thompson (3) has been adapted for this study.

METHOD OF STUDY

To study the incidence of urease-producing organisms in the oral flora unstimulated salivary samples were collected from a total of 82 patients and inoculated into "carbamide media." The media used to test for presence of urease-producing bacteria was similar to that prepared by Laidley and modified by Thompson and Shulte (3) (peptone 2 gms., sodium chloride 5 gms., distilled water 1000 cc., 20 cc. of a 0.2% alcoholic solution of thymol blue). The media was adjusted to pH 6.8. Ten cc. of this media was poured into each tube and sterilized by heat. After sterilization, 0.5 cc. of a Seitz filtered 10% carbamide (C.P.) solution was added to each tube and incubated for 24 hours. If not contaminated, tubes were stored in a refrigerator until used. This media is very selective for bacteria able to split carbamide.

[1] Received for publication January 29, 1943.

To each tube of carbamide media 0.2 cc. of unstimulated saliva was added and incubated for 48 hours at 37°C. Tubes uninoculated with saliva were incubated with each test as a control. After incubation color changes were noted and the results divided into 4 groups: *Group I.* Active urease production tests in which enough ammonia was formed from carbamide to turn the indicator, thymol blue, blue or dark blue. Several chemical analyses indicated that at least 25 milligrams per cent of free ammonia were required to turn the tubes dark blue. *Group II.* Moderate urease production—tests in which the indicator turned light blue or greenish blue and contained between 15 and 25 mg.% of free ammonia. *Group III.* Slight urease production—tests in which the color produced was green or yellow green and 5 to 15 mg.% of free ammonia was present. *Group IV.* No urease production—no color changes occurred and chemical analysis failed to show presence of any free ammonia.

Most of the patients studied for urease producing organisms were dental students who were also being studied for dental caries activity by the following methods: First, total colony counts of *L. acidophilus* which developed in 48 hours after 0.1 cc. of a 1 to 5 dilution of saliva had been spread on a Kulp's tomato agar plate (pH 5) (technic of Hadley, Bunting and Delves (4)). Second, depression of pH and total titratable acidity produced in 4 and 24 hours in a mixture of 0.4 cc. of 1% glucose and 3 cc. of saliva (test developed by Wach (5)). Third, amount of powdered enamel dissolved in 4 hours by a mixture of stimulated saliva, enamel and glucose (Fosdick, Hansen and Epple (6)). These tests were compared with clinical and radiographic examinations for over 2 years and attempts made to determine the degree of the patient's caries activity (7).

RESULTS

A total of 405 tests on salivary samples from 82 patients were made using the carbamide media described above. Tabulation of results shows that 154 (38%) of the salivary samples fell in group I (active urease production) 111 (27.4%) were in group II, 111 (27.4%) were in group III and 29 (7.2%) were in group IV (No urease-producing organisms present). Thus, a total of 376 (92.8%) of salivary samples showed some degree of urease production. In 65.4% (groups I and II) the urease-production was in appreciable amounts.

Salivary samples taken from the same patient on different days often gave different results. Salivary samples taken 5 times in 1 day from each of 3 patients also showed some variations in urease production. However, the findings indicated a trend which was related somewhat with the acid production and the aciduric colony counts of the salivary samples.

Examples of the findings are as follows:

1. E. B., young adult male, age 23, clinically and by laboratory tests "dental caries inactive." Seven tests were made with carbamide media; 2 falling in groups I, 4 in group II, and 1 in group IV. Saliva from this caries-inactive patient contained many active urease producing bacteria in 6 of 7 tests.

2. A. H., young adult age 21, clinically and by laboratory tests slightly "caries active." Six tests made with carbamide media and all fell in group I.

3. M. T., young adult age 20, clinically and by laboratory tests slightly "caries active." Seven tests made with carbamide media, all in group I.

4. J. R., young adult age 23, clinically and by laboratory tests "caries inactive." Six tests made, 5 in group I and 1 in group III.

5. C. G., young adult age 22, clinically and by laboratory tests, "caries very active," 15 tests made, 5 in group I, 4 in group II, 4 in group III and 2 in group IV.

6. F. S., young adult, age 20, clinically and laboratory tests indicated caries to be "slightly active." 5 tests on carbamide media, 4 in group I and 1 in group III.

7. R. K., young adult age 20, clinically and laboratory test indicate "caries to be slightly active." Six tests on carbamide media, show 5 in Class II and 1 in Class III.

8. R. J., age 20, clinical and laboratory studies indicate "no caries activity." 6 tests made. 2 in class II and 4 in class III.

It will be noted that a high percentage of the tests showed some degree of urease production. Most of these tests were made on young adult students with fairly good oral conditions. Studies made on different groups of patients might give different results.

Attempts were made to correlate the incidence of urease-producing bacteria and dental caries activity as shown by the presence and action of aciduric and acidogenic bacteria. The following trends were noted. In most instances salivary samples containing active urease-producing organisms were almost entirely free of *L. acidophilus*, although many aciduric streptococci were often present. In salivary samples containing few urease-producing organisms *L. acidophilus* colony counts were usually very high. This correlation does not hold true if aciduric streptococci are considered, because high streptococci colony counts on pH 5 media were sometimes given by salivary samples which produced much urease.

Most salivary samples containing active urease-producing organisms also produced little acid by glucose fermentation, although this was not a consistent result. Similarly, samples which converted little carbamide produced much acid by glucose fermentation, although occasionally urease was produced from a sample of saliva which produced much acid and gave a high aciduric colony count.

In other words, tests of patients who were classified as "dental caries inactive" by clinical and laboratory studies usually showed active urease production; patients who were "caries-active" usually showed less urease production. It seems logical to assume that bacteria capable of forming urease might be important in producing a buffer for acids and hence be of some significance in dental caries. Our studies suggest a possible correlation between caries activity and degree of urease production, although the findings do not warrant a definite conclusion.

It was thought presence of urease in the mouth would result in an alkaline reaction which might favor dental calculus deposition. Comparisons of urease production by patients who were susceptible to heavy deposits of calculus and those free from calculus were inconclusive, but the study is being repeated in a larger and more critical experiment.

Attempts to isolate and identify the organisms were made in approximately 15 instances. In every test which showed marked urease production, one of the *Proteus* group was isolated. Streptococci and Staphlococci were also found,

particularly from tests falling in group II and group III. In tubes showing no urease production viable organisms usually could not be isolated.

Use of carbamide in a mouth rinse should result in its conversion to ammonium carbonate in most cases because of the presence of urease producing bacteria in the oral flora.

SUMMARY

Bacteria capable of producing urease were found in 92.8% of 405 salivary samples from 82 patients. In 65.4% of the samples, appreciable quantities of urease were produced.

In general, salivary samples which gave low *L. acidophilus* colony counts and a limited amount of acid production from glucose, contained more active urease-producing bacteria than did salivary samples which gave extremely high *L. acidophilus* counts and produced much acid.

Significance of these findings to the formation of dental caries and salivary calculus was not established, but deserves further study.

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STUDIES ON THE INCIDENCE AND CAUSE OF DENTAL DEFECTS IN CHILDREN[1]

I. Prophylaxis

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The value of oral hygiene in the prevention of dental ailments has long been debated. The teeth of primitive people, were reported to be free from caries in spite of their lack of attention to oral hygiene. The inhabitants of Tristan da Cunha, who were found practically free from dental decay, never brushed their teeth (1). The Melanasian population inhabiting the island of Manus paid no attention to mouth hygiene; nevertheless, 98 per cent of these people were reported to be free from caries (2). The pupils of the American Samoa, as reported by Ferguson (3), never exercised habits of oral hygiene, yet, 90 per cent of them showed immunity to caries. Allusion is often made to children inhabiting civilized countries who have been reported to be free from caries in spite of the fact that they seldom, if ever, brush their teeth.

Suk (4) expressed but little confidence in the value of tooth brushes and mouth washes. Those races that adhered to toothbrushing and mouth washing, he asserted, were the possessors of the worst teeth. In over 1000 natives of Africa he studied, he noted absence of film on the teeth, in contrast to that observed in the mouths of "over domesticated" people. Responsible for the dental superiority of the native African is the consumption of hard foods and the ensuing vigorous mastication, Suk inferred.

Studies carried out by Klatsky (5), on hundreds of patients over a period of many years, have convinced him that the determining factor in the control of caries is mastication.

He further emphasized that soft foods fail to stimulate sufficiently the muscles of mastication (6). As a result of this lack of proper function, an unsanitary condition of the mouth developes. Food debris accumulation, plaque formation, and bacterial colonization with its production of lactic acid follow and caries is initiated.

In Shelby County, Tennessee, McRae (7) investigated a group of 3188 white and 1096 colored pupils. The number of colored children using the tooth brush was found to be smaller than that of white pupils. In spite of this circumstance, the colored children had better teeth. Shelling and Anderson (8), who examined a mixed group of white and colored children, found that a lower incidence of caries occurred in mouths exhibiting a state of poor oral hygiene. Brodsky (9), from

[1] Received for publication July 16, 1942. (Revised by author December 3, 1942.)

his study of approximately 600 New York City children, concluded that oral hygiene by itself cannot be interrelated with the prevention or incidence of caries. It may, however, exert its influence in conjunction with other circumstances. Bibby (10) called attention to the fact that perfect teeth are noted, quite often, in the "dirtiest mouths". He suggested that because of this condition "one may raise a question" as to the possibility of harm from strongly adhering to prophylactic habits, when, as a matter of fact, it is impossible to completely remove the bacterial plaque. Hyatt (11) concluded that, as the bristles of the tooth brush are 3 to 5 times wider than fissures, oral hygiene is of little consequence in preventing caries.

An analysis of the views of those advocating oral hygiene is important for comparison with those above. Mabalay (12) found that clean teeth did not insure complete immunity to caries. However, he reported, "there are twice more unclean teeth that have dental caries than those that have none". Clements and Kirkpatrick (13), from their examination of 920 school and pre-school children of New South Wales, Australia, found a higher incidence of caries associated with a higher insufficiency of oral hygiene.

There appears to be a difference of opinion as to how often the teeth should be brushed. Hoesler and Fain (14) concluded that it is important to brush the teeth more than once a day. They maintained that this gave an additional 3-hour protection from "mouth acids". The subjects they studied were given bread to eat and the debris accumulated on their teeth was collected and tested for acidity. In those who had brushed their teeth 24 hours before, the debris showed distinct acidity within 2 hours while, in those who brushed their teeth a short time before, the debris became acid after 5 hours. That daily use of the tooth brush injures the dental enamel is believed by many. In some measure these fears appear to be justified. In Stockholm, with a mechanism specially built for the purpose, Naeslund (15) brushed 12 newly extracted teeth without interruption for a term corresponding to the employment of the tooth brush twice daily for close to 50 years. He found that "light pressure with water or a chalk paste" did not impair the quality of the enamel. On the other hand, heavy-pressure brushing resulted in substantial wear. Consequently, it seems a fair inference that light pressure be advocated when brushing the teeth. Allan (16), who carried out field work among school children of Wakefield, England, observed that "frequent use of the tooth brush is not a guarantee against dental caries, but it is an established fact that a clean tooth is not so likely to decay as an unclean one".

Brushing the teeth, Mellanby (17) suggested, may bring about more harm than good. She stated that the tooth brush is rarely a clean instrument and that an improvement in appearance appeared to be the only gain resulting from its use. Mellanby's convictions on the value of vitamin D in the prevention of caries have received world-wide attention. The impartial observer, however, should be ready to agree that the use of a "dirty" tooth brush has never been sanctioned. In its simplest form "hygiene" stipulates cleanliness.

In Morgan's (18) conception, if a patient strictly adhered to instructions per-

taining to oral health and returned every 3 months for periodical examination, "oral hygiene can and will reduce the ravages of dental decay". He also contended that the occurrence of caries in mouths that are kept clean may be due to some glandular disturbance.

A number of investigators, while convinced of the value of oral hygiene, decline to recognize the merits of the tooth brush. They do so with conviction. Thus, Wallace (19) maintained that fibrous foods help "to keep the teeth cleaner than a tooth brush can keep them". Chewing food of this type effects dislodgement of food-particle accumulations in the crevices of the teeth and the embrasures. Unless such collections are removed, they become sources for the initiation of caries. Wallace (20) believed that dental decay is the result of carbohydrate stagnation which forms the basis of lactic acid fermentation. Such stagnation, he found is induced by the consumption of soft foods and the subsequent retention of the sticky pabulum about the teeth. This appears true in a degree, yet experimental evidence is needed to establish conclusively that detergent foods prevent, reduce, or remove debris accumulations. Through observations carried out by Wishart (21) on school children of Wakefield, England, it was shown that a reduction in carbohydrate stasis followed regular daily use of the tooth brush. Carque (22) suggested that the chewing of hard and tough food masses anatomically brushes the teeth. White and shiny teeth, she declared, are reported by explorers in uncultured countries whenever the natives thrived on such nutriments. Critical students will agree that Carque's conclusion stands on a rather frail basis.

RELATION BETWEEN ORAL HYGIENE AND CARIES

The deductions to follow have been formulated from study of 5103 public-school pupils of Newark, New Jersey. Six hundred and twelve were colored (285 girls and 327 boys). Various nationalities and social strata were included. Their ages ranged from 5 to 15 years. The group was divided into 3 subgroups: First, those who presented "clean" teeth; second, those whose teeth were "fair"; and third, those whose teeth were filthy or "dirty". It was considered best that a study of the relationship of "state of teeth" to caries be limited to the first and third subgroups, namely, those exhibiting clean teeth (free from stains and food) and those displaying dirty teeth (with stains and food on teeth abundantly revealed). Two thousand, four hundred and sixty-six children comprised these 2 groups.

To determine a possible relationship between oral hygiene and the presence of defects, it was considered correct to declare a mouth in a state of good health if, at the time of examination, no active caries necessitating repair was exhibited. The mouth mirror, explorer, college pliers, cotton pellets and scalers were the instruments employed in the accumulation of the data assembled. The x-ray was not utilized as an adjunct.

The fundamental fact brought to light was that the subgroup of pupils showing clean teeth included a larger number of subjects who were free from active dental decay. Of the 1063 white girls with clean teeth, 30 per cent did not show the presence of active caries (Table I), but only a little over 4 per cent of the 42

TABLE I

Clean teeth and the incidence of active caries

	WHITE		COLORED	
	Boys	Girls	Boys	Girls
No. of pupils with clean teeth	901	1063	142	168
No. with clean teeth and active caries	604 (67.0%)	743 (69.8%)	96 (67.6%)	113 (67.2%)
No. with clean teeth and no active caries	297 (33.0%)	320 (30.2%)	46 (32.4%)	55 (32.8%)

TABLE II

Dirty teeth and the incidence of active caries

	WHITE		COLORED	
	Boys	Girls	Boys	Girls
No. of pupils with dirty teeth	129	42	12	9
No. with dirty teeth and active caries	113 (87.6%)	40 (95.2%)	10 (83.3%)	5 (55.5%)
No. with dirty teeth and no active caries	16 (12.3%)	2 (4.8%)	2 (16.7%)	4 (44.5%)

TABLE III

Use of the tooth brush. Number of pupils classified in each subgroup

	WHITE		COLORED		TOTAL
	Boys	Girls	Boys	Girls	
No. of pupils examined	2140	2102	321	285	4848
No. of pupils who brushed their teeth daily	1043	1312	149	173	2677
No. of pupils who brushed their teeth occasionally	963	715	147	90	1915
No. of pupils who never brushed their teeth	134	75	25	22	256

TABLE IV

Use of the tooth brush. Percentage of children free from caries in subgroups

	NUMBER OF STUDENTS	NO. STUDENTS WITH NO ACTIVE CARIES	PER CENT STUDENTS WITH NO ACTIVE CARIES
Brushed teeth daily	2677	796	29
Brushed teeth occasionally	1915	339	17
Never brushed teeth	256	36	14

white girls exhibiting dirty teeth failed to display dental decay (Table II). While 33 per cent of the white boys with clean teeth showed no active caries (Table I), only a little over 12 per cent of those with dirty teeth evidenced no sign of the disease (Table II). Of the colored girls with clean teeth, 32 per cent disclosed no active evidence of the ailment, but over 44 per cent of those with unclean teeth exhibited active cavities. Of the colored boys with clean teeth, over 32 per cent were free from active caries, but 16 per cent of those with dirty teeth failed to display caries.

The white girls with clean teeth were somewhat less susceptible to caries than the white boys in this subgroup, but the percentage of white boys with dirty teeth who escaped dental decay was higher than that of the white girls belonging to this subgroup. Of the colored boys and girls with clean teeth, the resistance to caries was nearly equal in both sexes, but the colored boys with dirty teeth displayed a lower resistance to caries than did the girls. Comparing the relationship of clean and dirty teeth to caries in the white and colored girls, a slightly higher percentage of white girls with clean teeth exhibited dental decay. The percentage of white girls with dirty teeth who showed no signs of caries was far below that of the colored girls. The percentage of white boys with clean teeth who did not show caries was nearly similar to that of the colored boys, but a larger percentage of colored boys with dirty teeth appeared to be immune to caries. In recent years no investigator has claimed for clean teeth complete immunity to caries. But that clean teeth are less subject to the disease appears amply evident.

The findings here presented show that, where a state of good dental health has been established through reparative work or natural resistance to caries, its continuance is more definitely associated with clean than with dirty teeth. Tables I to IV present data in support of this conclusion.

RELATION BETWEEN USE OF TOOTH BRUSH AND CARIES

The value of the tooth brush as an implement contributory to the prevention of caries has been questioned by many in the profession. Its influence on the removal of food debris from the various surfaces of the teeth has failed to receive recognition from many authorities. Some convincing light on the theme under discussion will be shed by the present report on 4848 pupils. As 255 of the total of 5103 examined could not be correctly classified, it was considered best to eliminate them from the present study. Those investigated were segregated into 3 divisions: 1. those who brushed their teeth daily; 2. those who brushed their teeth occasionally; 3. those who never brushed their teeth. The information pertaining to brushing habits was obtained by questioning the child at the time of examination. A check-up as to the validity of the answers was undertaken by the teachers of a number of classes at a later date when the pupils were unexpectedly questioned again. The replies were found to coincide in nearly every instance. A basis for the veracity of the answers accumulated was thus established (Table III).

Of those examined 2677 brushed their teeth daily. Of these, a little over 29 per cent were free from active caries. Of the 1915 pupils who brushed their

teeth occasionally, 17 per cent did not disclose the presence of cavities. Of the 256 children who never brushed their teeth, a fraction over 14 per cent did not reveal carious teeth (Table IV). The inhibitory influence of home care on caries has thus been shown.

The percentage of white girls who brushed their teeth daily, but had active caries, exceeded that of the white boys belonging to this group. In the case of the colored pupils who exercised this habit day by day, the percentage of those who had dental decay was nearly equal in both sexes. The great problem which confronts us, then, is to learn what conditions contribute to an increased predisposition to caries in the white girls. The percentage of white boys and girls who comprised this group and showed the ravages of the disease exceeded that of the colored boys and girls, thus indicating that race or racial environment has an influence on the incidence of dental decay.

Of the group who brushed their teeth occasionally, more white girls than boys had carious teeth but of the colored pupils more boys than girls exhibited caries in this subgroup. In this group, also, the white pupils who brushed their teeth occasionally showed a larger percentage of carious teeth than the colored pupils.

Of the children who never brushed their teeth, the percentage of white boys who showed defects was slightly higher than that of the white girls. In the case of the colored children making up this division, the percentage of boys showing carious teeth far exceeded that of the girls.

Only a slightly higher percentage of white girls than colored girls, who did not brush their teeth, showed evidence of the disease. On the other hand, the number of colored boys who never utilized a tooth brush but had carious teeth exceeded that of the white boys. I am convinced that the play of forces producing caries is at times so complex that individualism of race cannot withstand its attacks with superior resistance.

The fundamental fact revealed in this study was that more pupils were free from active caries in the group who brushed their teeth daily than in the group who attended to this procedure occasionally or in those who never did.

SUMMARY

This study of 5103 grade school children of Newark, New Jersey revealed that a state of good dental health, established through reparative work or natural immunity, is more definitely associated with clean than with dirty teeth. A study of tooth brushing habits established the fact that more children were caries free among those who brushed their teeth daily than among the groups that used the tooth brush occasionally or not at all.

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STUDIES ON THE INCIDENCE AND CAUSE OF DENTAL DEFECTS IN CHILDREN[1]

II. Hypoplasia

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Hypoplasia of the teeth is regarded by many as one of the principal factors in predisposition to caries. The term denotes an imperfect formation or development. For a long time many investigators have believed that the quality of the structure of the tooth is directly responsible for caries immunity or susceptibility. It is obvious that if hypoplasia is an important factor in the incidence of caries, the extent of its occurrence and the nature of its causes should be considered.

Milligan (1), who investigated 5757 children attending the Norfolk schools in England, found that 258 of those studied disclosed hypoplasia of the permanent teeth and 9 exhibited hypoplasia of the deciduous teeth. Day and Sedwick (2) in their examination of 453 children in Rochester, New York, found 33 individuals exhibiting hypoplasia and 432 of the group with caries. Clements and Kirkpatrick (3) in New South Wales, Australia, found 12 per cent of 920 children afflicted with hypoplasia and, over 92 per cent of the 920 with carious teeth. Sterling (4) who has examined over 5000 negro children in Atlanta, Georgia, found that hypoplasia occurred in 2.5 per cent, and caries in 69 per cent of those studied. In a study of 34 children who presented generalized hypoplasia, Brodsky (5) found that defective structure alone does not necessarily induce dental decay. The caries incidence in his group was remarkably low (0.44 cavities per child). Wallace (6) reported that Hutchinson's teeth, regardless of their poor calcification, rarely decay. In England, he stated, the lower incisors are hypoplastic in 10 per cent of the people, yet, in the second permanent molars in which hypoplasia is practically unknown caries occurred 20 times more frequently than in the lower incisors.

The preceeding data refer chiefly to the incidence of gross hypoplasia and its relationship to the occurrence of caries. Mellanby (7) found, in her microscopic and macroscopic studies, 85 per cent of 302 deciduous teeth from 218 children hypoplastic; over 12 per cent moderately hypoplastic; and only 3 per cent normal. Of the 255 teeth found hypoplastic 253 were carious. Therefore, according to her impressions, a direct relationship between hypoplasia and caries was established. But, according to Shaw (8), Pitts, at the Royal Hospital of London, studied microscopically 5000 deciduous teeth and of these, less than 1 per cent were affected with hypoplasia, in striking disagreement with the Mellanby concept of hypoplasia incidence and her theory of its relationship to the initiation of caries.

McCall classified enamel defects of the deciduous teeth as "external" or hypo-

[1] Received for publication June 16, 1942. Revised by author December 3, 1942.

plasia and "internal" or hypocalcification. In the first type, deformities of structure are discernible to the naked eye; in the second, the outer formation of the tooth is normal but the quality of the structure is deficient (9). Observations conducted at the Guggenheim Clinic revealed that such hypocalcification of deciduous teeth is more likely to result in caries "than hypoplasia of the permanent teeth", in which the surface configuration is disturbed but the quality of the enamel is compact (10). McCall's conclusion cannot be ignored but it does not appear to be rigorously demonstrable.

In Wallace's (11) estimation hypoplasia resulted from an interference with the normal function of the enamel forming cells while the enamel is being deposited. This disturbance may be brought about by such diseases as measles, mumps, scarlet fever, whooping cough and others. Gardner (12) shared the same view and added that, generally, it is that segment of the tooth that was in its formative stage at the time the disorder occurred that is affected. Ruppe and Hénault (13) associate hypoplasia, in many instances, with congenital syphilis. Eliot, Souther, Anderson and Arnim (14), Shelling and Anderson (15), McKay and Rose (16) and others related its existence to early-life rickets. That faulty nutrition is involved in its occurrence was stressed by Marshall (17). The results of his studies have warranted such belief.

Milligan (1) contended that bottle feeding is one of the most important influences responsible for its development. He found that not one of those affected, on whom he obtained definite data, was breast fed. He also included, canned foods among the etiological factors. He found the condition to be more prevalent among the children of the poor laboring classes. However, according to Bunting (18) hypoplasia also occurs in children of "preferred families". Such were also my observations.

The relationship of endocrine disturbances to the causation of many dental ills is significant. Fleischman (19) reported that children afflicted with tetany presented hypoplasia of the enamel. This type of defective development manifested itself in the formation of horizontal furrows on the surfaces. Later, that insufficiency of the parathyroid secretion in childhood is responsible for hypoplastic enamel was demonstrated to other investigators in the field. Thus, Barker (20) called attention to hole-like imperfections and horizontal grooves in hypoparathyroidism. My studies on this subject relate hypoplasia to endocrine hyposecretions. Engelbach (21) associated hypogonadism with hypocalcification of the tooth substance. I have noted excessively defective calcification of the enamel in the upper second incisors and canines of children afflicted with deficient secretory activity of the gonads (testes and ovaries) (22). According to Bolt (23) hypoplasia of the enamel may be the result of a deranged equilibrium in the thyro-parathyroid mechanism.

DATA

Below, the extent of hypoplasia distribution among a group of 1921 Newark, New Jersey, public school pupils is reported. Its relationship to caries in these pupils will also be indicated.

Nine hundred and ninety boys and 931 girls were studied. Included among

them were 35 negro pupils (17 boys and 18 girls). Their ages ranged from 4 to 16 years. Their socio-economic status was above average. Nineteen nationalities were represented (Table I).

The study of the incidence of dental hypoplasia in the subjects under consideration included a careful macroscopical examination of the crowns of the teeth supplemented by a cautious interpretation of the deficiencies discovered. The mouth mirror, explorer, college pliers, scalers and cotton pellets were the instruments and accessories utilized in the procedures. The x-ray was not employed as an adjunct in diagnosis. This study, therefore, limits itself to an investigation of gross hypoplasia and its relationship to caries incidence.

Teeth were judged as hypoplastic when their coronal surfaces presented deeply or lightly pitted or furrowed appearance conveying a definite impression of a disorganized process of calcification. Some of the teeth so classified were so devoid of enamel as to have markedly defective configurations. Thus, in some of the permanent first and second incisors, this malformation occurred either in the lower third or half of the labial surfaces, or took the effect of either smooth or pitted grooves traversing the middle portion of these surfaces. Instances were noted where 2 rows of furrows were present, one crossing the gingival, the other, the incisal third of the labial portions of the crowns. In some cases also the mesial and distal aspects of these teeth were slightly involved. The permanent canines, when thus affected, revealed the incisal third of their labial surfaces as the seat of this deformity. Here, too, the mesial and distal aspects of these teeth appeared to be slightly included. The premolars and molars (of the latter, both permanent and deciduous) were occlusally defective or deformed. Some presented an almost complete absence of cusps. An involvement of the buccal areas was noted in a number of them. Marked disturbances of form in the deciduous first and second incisors were absent. The canines revealed promiscuous pitting or deformations on the incisal third of their labial aspects.

Of a total of 28,934 permanent teeth examined, 216 (0.7%) were hypoplastic (Table II). Of these, 44 exhibited caries and 172 were free from caries. The permanent teeth that had dental decay (2,313) were nearly 11 times more numerous than those having hypoplasia). The influences that cause such variations in the 2 disorders obviously cannot have a common origin, and it is concluded that there is no relation between hypoplasia and caries.

The permanent teeth most frequently affected were the upper first incisors. The lower first permanent molars, the upper first permanent molars, the lower first incisors, the lower second incisors, the upper and lower canines, the upper and lower second molars, and the upper and lower premolars followed in this order (Table IV).

Of the 75 first incisors declared hypoplastic, only 2 upper were found carious. In the case of the first permanent molars, 39 out of the 70 having the defect also exhibited decay. However, of the total number of first permanent molars examined (6,195) only 70 were hypoplastic. Caries was found in over 25 times as many of these teeth, (1,755), a further unmistakeable indication of the lack of association between the two anomalies (Table II).

Sex appeared to play little part in the distribution of hypoplasia. Thirty-nine

TABLE I

Incidence of hypoplasia in the boys and girls studied

	WHITE BOYS	WHITE GIRLS	NEGRO BOYS	NEGRO GIRLS	TOTAL
Number of pupils examined	973	913	17	18	1921
Pupils with hypoplasia	39 (4%)	35 (3.8%)	0	3 (16.6%)	77 (4.0%)

TABLE II

Permanent teeth. Incidence of hypoplasia with and without concurrent caries in white boys and girls and negro girls

	White boys														White girls												
	UPPER														UPPER												
	7	6	5	4	3	2	1	1	2	3	4	5	6	7	7	6	5	4	3	2	1	1	2	3	4	5	6	7
With caries		5											5			3		1		1	1	1	1				3	
Without caries		5			3	1	9	8	2	3			4		1	3			3	2	8	8	2	3		1	3	1
	LOWER														LOWER												
With caries		6											5			4											4	
Without caries		3	1		3	9	10	10	7	4			3		1	1			3	5	6	6	5	2		1	5	1
	Negro girls																											
	UPPER																											
With caries		1											1															
Without caries		1						2	2				1															
	LOWER																											
With caries		1											1															
Without caries		1				2	2	2	2				1															

TABLE III

Deciduous teeth. Incidence of hypoplasia with and without concurrent caries in white boys and girls

	White boys										White girls									
	UPPER										UPPER									
	E	D	C	B	A	A	B	C	D	E	E	D	C	B	A	A	B	C	D	E
With caries	1	1	1		1	1		2	1	1	1		1	1				1		
Without caries	4	4	4					5	7	4	2	6	2					2	8	1
	LOWER										LOWER									
With caries									1	1	3	1	1					1	2	1
Without caries	5	7	3					3	6	3	3	6	3	1			1	1	9	6

boys and 38 girls (4%) of the total number examined, comprised those affected (Table I). The 2 permanent hypoplastic first incisors attacked by decay belonged to the female group. The boys exhibited a preponderance of hypoplastic first permanent molars, and these hypoplastic teeth showed a larger incidence of caries than in the girls.

TABLE IV

Incidence of hypoplasia in the permanent dentition

		NUMBER OF TEETH EXAMINED	TEETH WITH HYPOPLASIA
Upper	2nd Molars	848	2 (0.23%)
	1st Molars	3180	35 (0.97%)
	2nd Premolars	1378	1 (0.07%)
	1st Premolars	1650	1 (0.06%)
	Canines	1270	12 (0.93%)
	2nd Incisors	2548	9 (0.35%)
	1st Incisors	2986	39 (1.17%)
Lower	2nd Molars	1093	2 (0.18%)
	1st Molars	3015	35 (1.02%)
	2nd Premolars	1316	2 (0.14%)
	1st Premolars	1676	0 (0.00%)
	Canines	1745	12 (0.67%)
	2nd Incisors	2893	30 (0.89%)
	1st Incisors	3326	36 (1.08%)

TABLE V

Incidence of hypoplasia in the deciduous dentition

		NUMBER OF TEETH EXAMINED	TEETH WITH HYPOPLASIA
Upper	2nd Molars	2252	14 (0.62%)
	1st Molars	1908	27 (1.3%)
	Canines	2351	18 (0.76%)
	2nd Incisors	980	1 (0.10%)
	1st Incisors	507	2 (0.39%)
Lower	2nd Molars	1960	22 (0.11%)
	1st Molars	1721	32 (0.18%)
	Canines	1883	12 (0.63%)
	1st Incisors	772	2 (0.25%)
	2nd Incisors	401	0 (0.00%)

None of the 17 negro boys showed signs of hypoplasia. However, 3 of the 18 colored girls were among those affected; of 20 hypoplastic teeth, noted in them, 4 were carious (2 upper and 2 lower first permanent molars).

Of a total of 14,735 deciduous teeth examined, 130 (0.88%) were hypoplastic (Table III). Of these, only 24 had caries. The balance (106) showed no sign

of caries. The deciduous teeth that had caries (3,057) were 23 times more numerous than those having hypoplasia. The difference is so striking that one is forced to conclude that hypoplastic derangements in these organs cannot be related to the initiation of caries.

Of the deciduous teeth, the first molars were most generally affected. Next in order were the second molars, and the upper and lower canines. The incisors furnished a negligible number (Table V). The number of hypoplastic deciduous teeth was nearly equally divided among the boys and girls, 66 in the boys, 64 in the girls. Similarly, the incidence of decayed hypoplastic deciduous teeth was nearly equal in both sexes (11 in the boys, 13 in the girls). Only 1 hypoplastic tooth occurred in the colored female group. This was free from dental decay. The preponderance of hypoplastic teeth free from caries was marked.

The incidence of hypoplasia in Sterling's group of colored children was 2.5 per cent. The present group of white pupils revealed this defect in 4 per cent. Active caries occurred in 70 per cent of these pupils and in 69 per cent of Sterling's group. Racial differences were devoid of significant influences.

CONCLUSIONS

From the evidence of these findings and the data submitted by other investigators as previously indicated, it is evident that there is no relationship between caries and such structural defects as hypoplasia. The incidence of hypoplasia is low, and in no way compares with that of dental decay.

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A NEW MICRO-METHOD FOR THE DETERMINATION OF FLUORIDES[1]

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The standard technique (1, 2) employed in the quantitative analysis of fluorine, presents certain difficulties. It involves a lengthy distillation, in which losses of fluorine may occur (3). The method further requires the titration of the suitably buffered distillate with thorium nitrate, using sodium alizarin sulphonate as the indicator. The analyst may find the end-point difficult to detect (4). These difficulties can be minimized by the use of a different method in the preparation of the fluoride solution. This is accomplished by combining receiver and flask and by concentrating the only source of moisture on the thorn of a modified Feigl distillation flask (*fig. 1*). The Feigl flask has been previously used in the qualitative detection of fluorine (5).

The addition of indigo carmine to the sodium alizarin sulphonate indicator, in the titration with thorium nitrate, produced a very reliable indicator (6). The solution, upon titration, changes from a green color to gray or colorless, and with further addition of thorium nitrate, a violet color appears. The color changes are definite. A standard may be taken at any point in the gray range, as well as at the first appearance of the violet color. It is helpful to use both end-points, the second being a convenient check on the first.

By these methods, the accurate quantitative determination of fluoride in the range of 1.0 micrograms to 10.0 micrograms of fluorine were made with accuracy, as the experimental data below indicate.

METHOD

Place sufficient quartz sand, approximately 40 mesh, in the lower portion of the distilling apparatus, so that a space of at least 7 mm. is maintained between the surface of the sand and the bottom of the thorn. Add the sample. If the sample is fluid, place lower portion of apparatus in an oven at a temperature of 96°C. and evaporate to dryness (If sample is dry mix with quartz sand). Remove and cool for 5 minutes. Suspend a drop of water on the thorn of the stopper with a capillary tube. Add concentrated sulfuric acid, sufficient to cover the quartz sand in the lower portion of the distilling apparatus, taking care not to wet the walls of the tube. Immediately insert stopper into flask.

Heat the contents slowly for about 3 minutes with a microburner using a small flame. The burner is moved continuously during the heating process. The temperature should be maintained below the point where white fumes appear.

[1] Received for publication April 27, 1942. Revised by author February 23, 1943.

[2] Part of this work was done at the Guggenheim Dental Clinic, New York.

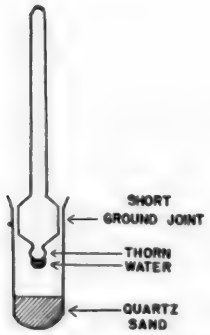

FIG. 1. Modified Feigl flask (½ actual size)

TABLE I

Titration of standard fluorine solution with approx. 0.0004 N thorium nitrate

FLUORINE	THORIUM NITRATE ADDED	AVERAGE	TITRATION VALUE MINUS BLANK	FLUORINE EQUIVALENT OF THORIUM NITRATE*
Gray end-point (0.38 cc.)†				
gamma	*cc.*	*cc.*	*cc.*	*gamma*
0.45	0.46	0.45	0.455	0.075	6.0
0.9	0.51	0.52	0.515	0.135	6.7
1.4	0.58	0.60	0.590	0.210	6.7
2.3	0.72	0.71	0.715	0.335	6.9
4.5	1.05	1.07	1.06	0.68	6.6
6.8	1.39	1.41	1.40	1.02	6.7
9.0	1.81	1.79	1.80	1.42	6.3
Average					6.6
Violet end-point (0.47 cc.)†					
0.45	0.54	0.54	0.54	0.07	6.4
0.9	0.62	0.61	0.615	0.145	6.2
1.4	0.67	0.69	0.68	0.21	6.7
2.3	0.81	0.81	0.81	0.34	6.8
4.5	1.16	1.18	1.17	0.70	6.4
6.8	1.51	1.52	1.515	1.05	6.5
9.0	1.89	1.87	1.88	1.41	6.4
Average					6.5

* The number of gammas of fluorine per cc. of thorium nitrate solution.

† The blanks obtained by the extrapolation of "best" lines to zero fluorine (y-axis) were 0.38 and 0.47 for the gray and violet endpoints respectively. These were used in the calculation of the data in this column.

Allow to cool completely. Wash thorn carefully into a 15 cc. centrifuge tube, diluting to 1 cc. To this solution add 0.1 cc. of indicator and 0.05 cc. of the buffer solution. The titration is made with 0.0004 N solution of thorium nitrate.

The end point is determined by comparing with a standard consisting of a solution of 10 gamma of sodium fluoride which has been prepared as above and titrated with thorium nitrate to an end point in the gray range.

As a check a second solution of 10 gamma of sodium fluoride is titrated to the violet color change. These are used as standard colors for the titration of the samples and must be prepared freshly. Either titration value can be used in calculating the fluorine content of the unknown solution.

TABLE II

Distilled samples of known solution

STANDARD SOLUTION FLUORIDE (10 PARTS PER MILLION)	0.0004 N THORIUM NITRATE TO OBTAIN GRAY ENDPOINT		TITRATION VALUE THORIUM NITRATE MINUS BLANK (0.38 CC.)		AMOUNT OF FLUORINE RECOVERED		LOSS
		Average		Average		Average	
gamma	*cc.*	*cc.*	*cc.*	*cc.*	*gamma*	*gamma*	*per cent*
0.9	0.510		0.130		0.85		5
	0.510	0.512	0.130	0.132	0.85	0.87	5
	0.515		0.135		0.89		1
2.3	0.715		0.335		2.21		4
	0.710	0.712	0.330	0.332	2.17	2.19	6
	0.715		0.335		2.21		4
	0.705		0.325		2.14		7
4.5	1.055		0.675		4.45		1
	1.055		0.675		4.45		1
	1.060	1.056	0.680	0.676	4.48	4.46	1
	1.050		0.670		4.42		2
	1.055		0.675		4.45		1
	1.060		0.680		4.48		1
6.8	1.410		1.030		6.79		1
	1.390	1.401	1.010	1.021	6.66	6.73	2
	1.400		1.020		6.73		1
	1.405		1.025		6.76		1
9.0	1.725		1.345		8.87		1
	1.735	1.731	1.355	1.351	8.94	8.91	1
	1.735		1.355		8.94		1
	1.730		1.350		8.91		1

The results obtained in analysis of aqueous solutions of sodium fluoride (sodium fluoride dissolved in distilled water) are given in Table II. The solution contained 10 gamma of sodium fluoride per cc.

PREPARATION OF SOLUTIONS

Indicator: 0.05 per cent sodium alizarin sulphonate and 0.0125 per cent indigo carmine in water. The maximum life of this solution is one week.

Buffer: Neutralize 50 cc. of 4M. monochloracetic acid to phenolphthalein with sodium hydroxide. Add 50 cc. more of 4 M. monochloracetic acid and dilute with water to 200 cc. This is the buffer used by Armstrong (1).

Thorium nitrate: Approximately 0.0004 N. The fluorine equivalent must be obtained for each batch of solution as shown in Table I.

DATA

Data (7) pertaining to the titrations of standard sodium fluoride solutions, ranging from 1 gamma to 10 gamma of fluorine, are listed in Table I. Before calculating the fluoride equivalent of the thorium nitrate solution, it is necessary

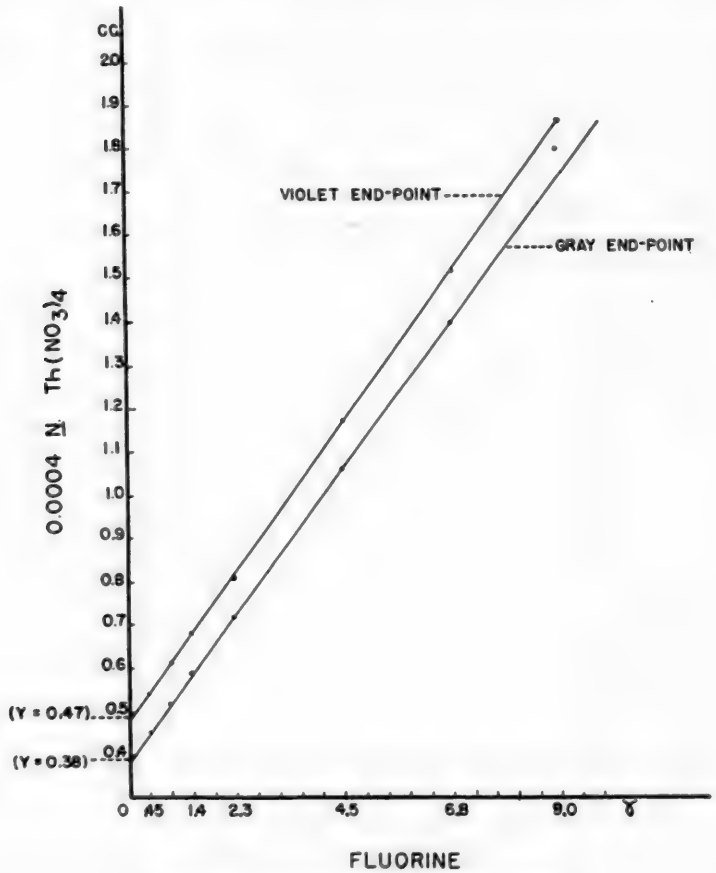

FIG. 2. Determination of titration blank

to determine the titration blank, which must be deducted from the reading. This is found graphically by extrapolation, as shown in *fig. 2*, based on the data in Table I. The intercepts on the ordinate represent the value of the blank.

SUMMARY

By this method, the accurate micro-quantitative determination of fluorine can be made in the range of 1.0 to 10.0 gamma of fluorine. The determination

of fluorine in this range may be done comparatively rapidly, simply and accurately, enabling the analysis of a large number of samples in a minimum of time.

Losses in fluorine, due to distillation and interference from glass and porcelain, have been minimized by combining receiver and flask in a modified Feigl tube.

The addition of indigo carmine to the sodium alizarin sulphonate indicator aids greatly in the determination of the end point.

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The author wishes to express his appreciation to Dr. W. D. Armstrong, University of Minnesota. The original manuscript was submitted, by the editor, to Dr. Armstrong who supplied data and suggested revisions of the method. This material has been incorporated by the author in this paper. The author also wishes to express his gratitude to Dr. Maxwell Karshan for his advice in preparing the manuscript.

Figs. 1 and 2 were prepared through the courtesy of Miss Jane Farwell.

FLUORIDE DOMESTIC WATERS AND DENTAL CARIES EXPERIENCE IN 2026 WHITE ILLINOIS SELECTIVE SERVICE MEN[1]

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Recent studies have pointed out an inverse relationship between the fluoride content of the public water supply and the dental caries experience of those children continuously using such waters throughout life (1). A similar relationship has also been reported (2) in a white adult male population composed of 487 selectees from 27 Illinois cities. These latter observations were divided into two major groupings consisting of (a) those who continuously used the public water supplies of varied fluoride content throughout life, and (b) those whose continuous exposure included the first 8 years of life only.

As it seemed apparent from these and other studies that fluoride levels between 0.0 and 1.0 p.p.m.[2] of F might markedly influence the intensity of dental caries attack, and/or the resistance of the teeth to caries attack, a further study was made of 2026 white selectees living in 91 Illinois communities (Table I) whose public water supplies contained fluorides of different concentrations. For purposes of classification these waters were grouped into one or the other of the following broad divisions:

FLUORIDE (F) CONCENTRATION OF DOMESTIC WATER IN P.P.M.	COMMENTS
0.0–0.1	Fluoride-free waters which in this particular study is limited to domestic waters obtained from either Lake Michigan or the Mississippi River.
0.5–0.9	Fluoride waters capable of influencing dental caries experience but which from the standpoint of maximum dental caries inhibition are suboptimal.
1.0 and over	Under average conditions in Illinois, a concentration of about 1.0 p.p.m. of F or slightly above may be considered the optimal level for dental caries inhibition. At levels above 1.5 p.p.m. the dental caries experience remains low but the beneficial effects from the standpoint of dental hygiene begin to be counteracted by the complicating factor of mottled enamel, a problem warranting increasing consideration as the concentration rises above 1.5 p.p.m. of F.

In this paper, therefore, "fluoride-free areas" are defined as those communities whose public water supply contains fluorides in the 0.0–0.1 p.p.m. range; "suboptimal-fluoride areas," 0.5–0.9 p.p.m., and "fluoride areas," 1.0 p.p.m. or over.

[1] Received for publication January 7, 1943.

[2] p.p.m. = parts per million.

TABLE I

Analytical and geological data on public water supplies in 91 Illinois municipalities

MUNICIPALITY	COUNTY	POPULATION, 1940	FLUORIDE (F) CONTENT IN P.P.M.	PRINCIPAL AQUIFERS
‡Abingdon	Knox	3,218	2.2	St. Peter, Cambrian
†Aledo	Mercer	2,593	1.9	Niagaran, St. Peter
‡Alexis	Mercer	877	1.8	Niagaran, St. Peter
‡Alton	Madison	31,255	0.0	Mississippi River
†Arlington Heights	Cook	5,668	1.2	Niagaran
‡Atkinson	Henry	719	0.7	Doubtful
‡Aurora	Kane	47,170	1.0	Niagaran, St. Peter
‡Batavia	Kane	5,101	0.9	Niagaran, St. Peter, Cambrian
†Beecher	Will	742	0.7	Niagaran
‡Belleville	St. Clair	28,405	0.0	Mississippi River
‡Bement	Platt	1,466	0.7	Glacial Drifts
†Bensenville	Du Page	1,869	1.4	St. Peter, Dresbach
‡Berwyn	Cook	48,451	0.0	Lake Michigan
†Blue Island	Cook	16,638	0.0	Lake Michigan
‡Bradford	Stark	907	1.5	St. Peter
‡Bradley	Kankakee	3,689	1.1	Niagaran, St. Peter
*Buda	Bureau	734	1.4	Niagaran, St. Peter
‡Bushnell	McDonough	2,906	4.0	St. Peter
‡Cabery	Kankakee	290	1.2	Niagaran
†Calumet City	Cook	13,241	0.0	Lake Michigan
‡Canton	Fulton	11,577	2.1	St. Peter
*Cary	McHenry	707	0.9	Niagaran
‡Capron	Boone	487	0.6	Doubtful
*Carpentersville	Kane	1,289	0.9	Glacial Drifts
*Chatsworth	Livingston	1,036	0.7	Doubtful
‡Chenoa	McLean	1,401	2.2	St. Peter
*Chester	Randolph	5,110	0.0	Mississippi River
‡Cuba	Fulton	1,620	3.0	St. Peter
†Cicero	Cook	64,712	0.0	Lake Michigan
*Cullom	Livingston	500	3.2	St. Peter
†Dallas City	Hancock & Henderson	1,149	0.0	Mississippi River
†Danforth	Iroquois	362	2.6	No record
‡De Kalb	De Kalb	9,146	0.6	St. Peter, Cambrian
‡Depue	Bureau	2,296	0.9	St. Peter
†Des Plaines	Cook	9,518	1.6	St. Peter, Cambrian
‡Dolton	Cook	3,068	0.0	Lake Michigan
‡East Moline	Rock Island	12,359	1.5	St. Peter
‡East St. Louis	St. Clair	75,609	0.0	Mississippi River
‡Elgin	Kane & Cook	38,333	0.9	Questionable
‡Elmhurst	Du Page	15,458	2.0	St. Peter, Cambrian
†Erie	Whiteside	1,052	0.5	Doubtful
‡Evanston	Cook	65,389	0.0	Lake Michigan
‡Farmington	Fulton	2,225	2.0	St. Peter
‡Flanagan	Livingston	663	0.7	Glacial Drifts
‡Galesburg	Knox	28,876	1.9	Dresbach
†Galva	Henry	2,812	1.9	St. Peter, Niagaran
‡Geneva	Kane	4,101	1.0	Niagaran, St. Peter, Cambrian
‡Glencoe	Cook	6,825	0.0	Lake Michigan
*Glenview	Cook	2,500	0.9	St. Peter, Cambrian
‡Granite City	Madison	22,974	0.0	Mississippi River
*Granville	Putnam	1,038	1.1	St. Peter
*Homewood	Cook	4,078	0.9	Niagaran
‡Joliet	Will	42,365	1.4	St. Peter, Dresbach
†Kempton	Ford	259	3.0	Niagaran
‡Kewanee	Henry	16,901	0.9	Dresbach
‡Knoxville	Knox	2,241	2.2	St. Peter, Dresbach
†Lake Forest	Lake	6,585	0.0	Lake Michigan
‡Lemont	Cook	2,557	1.4	St. Peter, Dresbach

TABLE I—*Concluded*

MUNICIPALITY	COUNTY	POPULATION, 1940	FLUORIDE (F) CONTENT IN P.P.M.	PRINCIPAL AQUIFERS
*Leonore	La Salle	179	0.5	Glacial Drifts
‡Lockport	Will	3,475	1.3	St. Peter, Dresbach
‡Lombard	Du Page	7,705	0.9	No information
‡Lyons	Cook	4,960	1.0	St. Peter, Niagaran, Cambrian
†Maywood	Cook	25,648	1.6	St. Peter, Cambrian
†Milan	Rock Island	1,210	2.4	No casing record
†Minonk	Woodford	1,897	2.2	Niagaran, St. Peter
‡Moline	Rock Island	34,608	0.0	Mississippi River
‡Monmouth	Warren	9,096	1.7	St. Peter, Cambrian
†Oak Park	Cook	66,015	0.0	Lake Michigan
‡Odell	Livingston	927	2.9	Niagaran, St. Peter
‡O'Fallon	St. Clair	2,407	0.0	Mississippi River
‡Oglesby	La Salle	3,938	1.0	Niagaran, Dresbach
†Oswego	Kendall	978	1.3	St. Peter
†Princeville	Peoria	996	2.3	St. Peter
‡Quincy	Adams	40,469	0.0	Mississippi River
‡Riverside	Cook	7,935	1.8	Niagaran, St. Peter, Cambrian
‡Rock Island	Rock Island	42,775	0.0	Mississippi River
†Saunemin	Livingston	341	3.6	Niagaran
*Sibley	Ford	374	0.6	Doubtful
‡Spring Valley	Bureau	5,010	0.6	Doubtful
†Standard	Putnam	334	1.6	St. Peter
‡Strawn	Livingston	199	0.6	Doubtful
†Toluca	Marshall	1,433	1.5	St. Peter
*Toulon	Stark	1,230	2.2	St. Peter
†Venice	Madison	5,454	0.0	Mississippi River
‡Viola	Mercer	743	2.4	St. Peter
‡Waukegan	Lake	34,241	0.0	Lake Michigan
‡West Chicago	Du Page	3,355	0.7	Doubtful
‡Winnetka	Cook	12,430	0.0	Lake Michigan
†Woodhull	Henry	638	2.4	Niagaran, St. Peter
‡Wyoming	Stark	1,360	1.8	St. Peter
‡Zion	Lake	6,555	1.2	Niagaran, St. Peter

* Selectees—entire life.
† Selectees—first 8 years of life.
‡ Selectees—both entire life and first 8 years of life.

All selectees were examined by dental examiners on the local selective service boards *before the dental requirements were relaxed.* Examinations were recorded on the new dental examination work sheet developed by the Division of Dental Health Education, Illinois State Department of Public Health, in co-operation with the Selective Service System (3).

The same methods used in the study of the 27 cities in Illinois with respect to public water supplies, age, color, continuity of exposure, and other epidemiological factors discussed in detail in that report were followed in the study of the 91 communities which form the basis of this paper.

CLASSIFICATION OF DEFECTS AND RATES

In computing an index to show the amount of dental caries in these population groups it was decided to express the amount of dental caries in terms of dental caries experience (4); for example, *untreated dental caries* means teeth that were carious but could be restored; *extractions indicated* applies to all broken-down teeth, roots, and unrestorable teeth; *filled teeth* applies to all filled teeth free from

carious lesions; *missing* applies to teeth that had been extracted because of dental caries; *no caries experience* applies to all teeth present with no caries experience. In computing this index no single tooth is counted more than once even though one surface may show a carious lesion and another a filling.

When it is desired to express the dental caries experience in terms of a rate per 100 selectees, the sum of the first 4 aggregates referred to is divided by the number of selectees examined and the quotient multiplied by 100. Present dental caries experience is determined by totaling the number of teeth with untreated dental caries and extractions indicated (irrespective of the number of defects per tooth). Past dental caries experience is determined by totaling the number of filled and missing teeth. The total caries experience (present and past) is determined, then, by totaling the number of teeth with untreated dental caries, extractions indicated, filled, and missing.

These rates are computed on a tooth population of 28 teeth. The third molars are omitted since there are so many variations in this tooth not connected with dental caries, such as congenitally missing and extracted because of malposition, that its inclusion would be of doubtful validity.

CLINICAL FINDINGS

Table II shows the dental caries experience of 169 selected white selectees who were born and had lived their entire lives in 20 municipalities where the public water supplies contained 0.5–0.9 p.p.m. of fluorides (F), and 454 selected white selectees who were born and had lived their entire lives in 31 municipalities where the public water supplies contained 1.0 p.p.m. or more of fluorides (F).

In order to compare these results from the use of fluoride-bearing waters, a comparison group was chosen of 286 selectees living in 15 municipalities where the public water supplies are fluoride free (Lake Michigan and Mississippi River water) who could qualify as to residence in the same manner as the fluoride area group.

The limited sample in all groups made it necessary to classify by age grouping instead of single ages.

0.5–0.9 P.P.M. Fluorides (F). Here we find that the selectees who have lived their entire lives in areas where the public water supplies contained 0.5–0.9 p.p.m. have an untreated dental caries rate of 81 less; filled rate of 109 less; extractions indicated rate of 5 less; missing rate of 97 less; and no caries experience of 291 more than those living in areas where the public water supplies are fluoride free. When these rates are combined as present and past the 2 groups compare as follows: the selectees living in suboptimal fluoride-areas (0.5–0.9 p.p.m.) have a present rate of 164 and a past rate of 624, while the selectees living in fluoride-free areas have a present rate of 249 and a past rate of 830. The total dental caries experience for this group may be compared as follows: the selectees who were born and had lived in suboptimal-fluoride areas all their lives have a total rate of 788 as compared with 1079 for the selectees who had lived in fluoride-free areas their entire lives.

1.0 or More Fluorides (F). The selectees who had lived their entire lives in areas where the public water supplies contained 1.0 p.p.m. or more of fluorides

TABLE II

Summary of the dental caries experience, permanent teeth (excluding third molars) of selected 21-37-year-old white selectees living their entire lives or first eight years of life, but not entire life, where the public water supply contains varying amounts of fluorides (F)

FLUORIDES (F) CONTENT OF PUBLIC WATER SUPPLY	DURATION OF RESIDENCE	AGE	NUMBER OF SELECTEES EXAMINED	DENTAL CARIES EXPERIENCE, PERMANENT TEETH											
				Teeth with untreated dental caries (a)		Filled teeth (b)		Extractions indicated (c)		Missing teeth (d)		No caries experience		Total (a+b+c+d)	
				Number	Rate per 100	Number	Rate per 100	Number	Rate per 100	Number	Rate per 100	Number	Rate per 100	Number	Rate per 100
0.5–0.9 p.p.m. fluorides (F)	Entire life	21–24	78	73	94	282	362	35	45	112	144	1682	2156	502	644
		25–28	56	87	155	245	438	34	61	166	296	1036	1850	532	950
		Over 28	35	24	69	142	406	24	69	108	309	682	1949	298	851
		Total all ages	169	184	109	669	396	93	55	386	228	3400	2012	1332	788
1.0 p.p.m. and over fluorides (F)	Entire life	21–24	252	391	155	577	229	75	30	359	142	5654	2244	1402	556
		25–28	104	144	138	306	294	37	36	199	191	2226	2140	686	660
		Over 28	98	95	97	303	309	44	45	288	294	2014	2055	730	745
		Total all ages	454	630	139	1186	261	156	34	846	186	9894	2179	2818	621
Fluoride free	Entire life	21–24	190	374	197	944	497	112	59	483	254	3407	1793	1913	1007
		25–28	73	134	184	355	486	47	64	315	432	1193	1634	851	1166
		Over 28	23	34	148	145	630	12	52	131	570	322	1400	322	1400
		Total all ages	286	542	190	1444	505	171	60	929	325	4922	1721	3086	1079
0.5–0.9 p.p.m. fluorides (F)	First eight years	21–24	67	131	196	182	272	45	67	143	213	1375	2052	501	748
		25–28	41	22	54	129	315	8	20	96	234	893	2178	255	622
		Over 28	47	94	200	105	223	36	77	384	817	697	1483	619	1317
		Total all ages	155	247	159	416	268	89	57	623	402	2965	1913	1375	887
1.0 p.p.m. and over fluorides (F)	First eight years	21–24	202	305	151	602	298	65	32	267	132	4417	2187	1239	613
		25–28	152	234	154	583	384	55	36	334	220	3050	2007	1206	793
		Over 28	159	133	84	654	411	63	40	439	276	3163	1989	1289	811
		Total all ages	513	672	131	1839	358	183	36	1040	203	10,630	2072	3734	728
Fluoride free	First eight years	21–24	232	418	180	974	420	116	50	498	215	4490	1935	2006	865
		25–28	129	172	133	653	506	87	67	457	354	2243	1739	1369	1061
		Over 28	88	116	132	348	395	87	99	541	615	1372	1559	1092	1241
		Total all ages	449	706	157	1975	440	290	65	1496	333	8105	1805	4467	995

(F) have an untreated dental caries rate of 51 less; filled rate of 244 less; extractions indicate rate of 26 less; missing rate of 139 less; and a no caries experience

of 458 more than those living in areas where the public water is fluoride free. When these rates are combined as present and past the 2 groups compare as follows: the selectees living in the fluoride areas have a present rate of 173 and a past rate of 448, while the selectees living in fluoride-free areas have a present rate of 249 and a past rate of 830. The total dental caries experience for this group may be compared as follows: the selectees born and having lived in fluoride areas all their lives have a total rate of 621 as compared with 1079 for the selectees who have lived in fluoride-free areas their entire lives.

First 8 Years of Life

In order to determine the early effect of fluoride waters during the first 8 years of life when the enamel of the permanent teeth (excluding third molars) is calcifying, 1117 white selectees were chosen and grouped according to the fluoride content of the public water supply. The dental caries experience of 155 selected white selectees who were born and had lived the first 8 years of their lives, but not their entire lives, in 15 municipalities where the public water supplies contained 0.5–0.9 p.p.m. of fluorides (F), and 513 white selectees who were born and had lived the first 8 years of their lives, but not their entire lives, in 43 municipalities where the public water supplies contained 1.0 p.p.m. or more of fluorides (F). In order to compare these findings with selectees living in municipalities where the public water supplies are fluoride free (Lake Michigan and Mississippi River water), 449 white selectees were selected from 21 cities who had lived in fluoride-free areas the first 8 years of their lives, but who now reside elsewhere.

0.5–0.9 P.P.M. Fluorides (F). When the rates are combined as present and past these groups compare as follows: the selectees who have lived the first 8 years of their lives, but not their entire lives, in municipalities where the public water supplies contained 0.5–0.9 fluorides (F) have a present rate of 217 and a past rate of 670, while the selectees who have lived in fluoride-free areas have a present rate of 222 and a past rate of 773. Thus the selectees from suboptimal-fluoride areas have a present rate lower by 5 and a past rate lower by 103. The total dental caries experience may be compared as follows: the selectees who were born and had lived the first 8 years of their lives, but not their entire lives, in suboptimal-fluoride areas have a total rate of 887 as compared with 995 for the selectees who were born and had lived the first 8 years of their lives in fluoride-free areas.

1.0 P.P.M. or More Fluorides (F). The selectees who were born and had lived the first 8 years of their lives but not their entire lives, in municipalities where the public water supplies contained 1.0 p.p.m. or more fluorides (F) have a present rate of 167 and a past rate of 561, while the selectees who have lived in fluoride-free areas have a present rate of 222 and a past rate of 773. Thus the selectees from the fluoride areas have a present rate lower by 55 and a past rate lower by 212. The total dental caries experience may be compared as follows: the selectees who were born and had lived the first 8 years of their lives, but not their

entire lives, in fluoride areas have a total rate of 728 as compared with 995 for the selectees who were born and had lived the first eight years of their lives, but not their entire lives, in fluoride-free areas.

Table III compares and combines the dental caries experience rates of the 3 area groups (fluoride free, suboptimal fluoride and fluoride). Here we find both the suboptimal fluoride, and the fluoride area rates considerably lower than those of the fluoride-free area rates.

TABLE III

Comparison of the dental caries experience rates in the permanent teeth (excluding third molars) of 625 selected white selectees (fluoride areas) and 286 white selectees (fluoride-free areas) living in respective areas their entire lives

AGE	NUMBER OF SELECTEES EXAMINED			DENTAL CARIES EXPERIENCE, PERMANENT TEETH																	
				Teeth with untreated dental caries (a)			Filled teeth (b)			Extractions indicated (c)			Missing teeth (d)			No caries experience			Total (a + b + c + d)		
	Fluoride free	0.5 to 0.9 p.p.m.	1.0 p.p.m. and above	Fluoride free	0.5 to 0.9 p.p.m.	1.0 p.p.m. and above	Fluoride free	0.5 to 0.9 p.p.m.	1.0 p.p.m. and above	Fluoride free	0.5 to 0.9 p.p.m.	1.0 p.p.m. and above	Fluoride free	0.5 to 0.9 p.p.m.	1.0 p.p.m. and above	Fluoride free	0.5 to 0.9 p.p.m.	1.0 p.p.m. and above	Fluoride free	0.5 to 0.9 p.p.m.	1.0 p.p.m. and above
				Number per 100 selectees																	
21-24	190	78	252	197	94	155	497	362	229	59	45	30	254	144	142	1,793	2,156	2,244	1,007	644	556
25-28	73	56	104	184	155	138	486	438	294	64	61	36	432	296	191	1,634	1,850	2,140	1,166	950	669
Over 28	23	35	98	148	69	97	630	406	309	52	69	45	570	309	294	1,400	1,949	2,055	1,400	851	745
Total all ages	286	169	454	190	109	139	505	396	261	60	55	34	325	228	186	1,721	2,012	2,179	1,079	788	621

Comparison of the dental caries experience rates in the permanent teeth (excluding third molars) of 668 selected white selectees (fluoride areas) and 449 white selectees (fluoride-free areas) living in respective areas at least the first eight years of their lives but not their entire lives

21-24	232	67	202	180	196	151	420	272	298	50	67	32	215	213	132	1,935	2,052	2,187	865	748	613
25-28	129	41	152	133	54	154	506	315	384	67	20	36	354	234	220	1,739	2,178	2,007	1,061	622	793
Over 28	88	47	159	132	200	84	395	223	411	99	77	40	615	817	276	1,559	1,483	1,989	1,241	1,317	811
Total all ages	449	155	513	157	159	131	440	268	358	65	57	36	333	402	203	1,805	1,913	2,072	995	887	728

Prosthesis and Rejections

Table IV compares the prosthesis and rejections of 2026 selectees living in areas where the public water supplies contained varying amounts of fluorides (F). It was found the greater percentage of prosthesis as well as rejections were in the fluoride-free areas. More extensive prosthesis among the fluoride-free group of selectees was also observed.

TESTS FOR SIGNIFICANCE

Calculating the standard error (5) of the difference between the caries experience of the several groups studied by means of the formula $\sqrt{\frac{P \times Q}{N_1} + \frac{P \times Q}{N_2}}$, we find that the difference in caries experience between those individuals who have lived their entire lives in areas where the water contained no fluoride (F) and those who have lived where water contained from 0.5–0.9 p.p.m. fluorides (F) is 12.22 times the standard error. The difference between the fluoride-free group and the group living their entire lives in areas where the water contained 1.0 p.p.m. fluorides (F) or over is 24.92 times the standard error. In comparing the groups that have lived in these areas at least the first 8 years of their lives,

TABLE IV

Comparison of prosthesis and rejections of selectees living in areas where the public water supply contains varying amounts of fluorides (F)

CLASSIFICATION OF SELECTEES		NUMBER OF SELECTEES EXAMINED	PROSTHESIS				REJECTIONS							
			Crowns and bridges		Dentures (full and partial)		Qualified for limited military service				Disqualified for military service			
							Dental		Both dental and medical		Dental		Both dental and medical	
Entire life	First eight years of their lives but not their entire lives		Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
Fluoride free		286	35	12.2	12	4.2	10	3.5	0	0.0	5	1.7	0	0.0
	Fluoride free	449	59	13.1	16	3.6	18	4.0	1	0.2	13	2.9	2	0.4
0.5 to 0.9 p.p.m. fluorides		169	12	7.1	3	1.8	2	1.2	0	0.0	3	1.8	0	0.0
	0.5 to 0.9 p.p.m. fluorides	155	7	4.5	4	2.6	1	0.6	1	0.6	3	1.9	1	0.6
1.0 p.p.m. fluorides and over		454	40	8.8	2	0.4	0	0.0	0	0.0	1	0.2	0	0.0
	1.0 p.p.m. fluorides and over	513	31	6.0	7	1.4	5	1.0	0	0.0	6	1.2	0	0.0

but not their entire lives, it was found that the difference between the fluoride free and the 0.5–0.9 p.p.m. was 4.67 times the standard error, while the difference between fluoride-free group and those using 1.0 p.p.m. and above was 16.96 times the standard error. A difference of 3 standard errors is considered significant.

From these figures, we may say, that the difference in dental caries experience of selectees living in fluoride-free, suboptimal-fluoride and fluoride areas is almost certainly due to some factor other than chance.

AGE DISTRIBUTION

Since age distribution of the groups compared varied, the average ages were computed. For those living their entire lives in the areas studied, the average

ages were fluoride-free group 23.9 years; 0.5–0.9 p.p.m. fluorides (F), 25.7 years; 1.0 p.p.m. and above, 25.3 years. For those living at least the first 8 years of their lives, but not their entire lives, in the areas studied, the average ages were fluoride-free group 25.2 years; 0.5–0.9 p.p.m. fluorides (F), 26.4 years; 1.0 p.p.m. and above, 26.5 years. Since the average ages of the fluoride-free groups were less than those where water contained either suboptimal fluoride or fluoride of 1.0 p.p.m. or more, we would expect the difference in caries experience to be even larger if the average ages were equal.

CONCLUSION

A study of 2026 white adults lends further support to the fluorine dental caries hypothesis. The domestic waters used by these selectees were grouped into 3 broad divisions: *fluoride free* (0.0–0.1 p.p.m. of F), *suboptimal fluoride* (0.5–0.9 p.p.m.) and *fluoride* (1.0 p.p.m. and over). Individuals were classified according to whether they had used the domestic water in question (a) during their entire life, or (b) during the first 8 years of life only (period of dental calcification). A lower dental caries experience and a higher rate of teeth with no caries experience was observed in those using either type of fluoride water when compared with those using fluoride free domestic waters, the higher fluoride waters (1.0 p.p.m. or over) being associated with the lowest dental caries experience.

Other factors being essentially the same it is reasonable to assume that the marked differences in dental caries experienced by the several groups is attributable to the differences in the fluoride content of the domestic waters used.

Attention is also called to the lower percentage of selectees rejected for military service because of dental conditions and the lower percentage using various forms of dental prosthesis, particularly dentures, in those selectees from the fluoride water areas.

Grateful acknowledgement is made for the assistance rendered by Mr. Clarence W. Klassen, Chief Sanitary Engineer, Mr. James G. Weart, Chemist, of the Division of Sanitary Engineering, and Miss Marie Rodgers, Statistician, of the Division of Dental Health Education, Illinois State Department of Public Health.

Appreciation is also expressed to Lt. Colonel E. Mann Hartlett, State Medical Director of the Selective Service System, whose efforts did much to insure proper development of the Selective Service System program.

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FURTHER STUDIES OF THE CALCIUM AND PHOSPHORUS CONTENT OF RESTING AND ACTIVATED SALIVA OF CARIES-FREE AND CARIES-ACTIVE INDIVIDUALS[1]

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The purpose of the following investigation is to critically analyze again the claim of Karshan that caries-free individuals have significantly higher total calcium and inorganic phosphorus values in mixed saliva than have caries-active individuals. In a study of this question recently reported (7), we were unable to confirm Karshan's findings in a group of 90 CF[2] and 108 CA[3] persons. Karshan's latest report on this subject (12) offers various reasons for this difference, which were based on his earlier observations of 1936 (10) and 1939 (11). An attempt shall be made to clarify all of the essential points brought forth by Karshan in support of his thesis, since confirmation of a higher calcium or phosphorus level in CF individuals would have a profound effect on the general concept of the etiology of dental caries.

Karhsan's interpretation of our failure to find significant differences between CF and CA individuals may be summarized to the effect that it is due to " . . . . differences in experimental conditions." Those mentioned by this author were: differences in the concept of clinical criteria for caries-activity and freedom, time of collection of samples, fasting condition, method of preliminary treatment, and finally the use of resting or activated saliva.

In many investigations of the past the term "caries-freedom" has been used in different ways. Some authors spoke of caries-freedom only when there were no cavities and no fillings as established by clinical (mouth mirror and explorer) and roentgenographic examination. Others accepted individuals as being CF who had a number of fillings but no cavities at the time of the examination. These methods of designating CF individuals prove most unsatisfactory and leave a wide range of error because the naked eye, however careful and thorough one may be in making an intra-oral examination, in many instances fails to disclose incipient caries or a caries-susceptible state of the individual.

Variations in interpretation of CA cases by mirror and explorer have also been

[1] Conducted under grants made by the American Foundation for Dental Science, California State Dental Association, and the Research Board of the University of California.

Presented at the 21st General Meeting of the International Association for Dental Research, Chicago, March 13 and 14, 1943. Received for publication February 18, 1943.

[2] CF = Caries-free.

[3] CA = Caries-active.

found. Karshan, for instance, requires that patients in the active-caries group have " . . . . active decay apparently of recent origin, associated with definite disintegration of enamel, accompanied by softened dentin." It must be obvious to every clinician in dentistry that in a mouth which appears CF, caries activity can go on undetected right under the very eyes of the examiner and, on the other hand, in another mouth "with definite disintegration of enamel accompanied by softened dentin" caries-activity might have come to a standstill, temporarily at least, without the examiner being aware of it. If one prefers the terms caries-immunity and caries-susceptibility, it must be recognized that these states cannot be determined by the mouth mirror, explorer and roentgenogram alone, and for this purpose additional means must be employed. The salivary *Lactobacillus acidophilus*[4] test has proved a most important aid in segregating these two distinct groups: one which satisfies all qualifications of caries-freedom by dental and roentgenographic examination with no previous fillings and with repeatedly negative *L.a.* tests before and at the time the saliva samples are taken for calcium and phosphorus analyses; the second group must be selected on the basis of rampant caries activity as expressed by new and old enamel and dentin caries on any surface of the teeth as well as repeatedly high *L.a.* counts before and at the time of sampling for calcium and phosphorus analyses.

The selection of CF and CA individuals for the previous report (7) was based on the above concept. It differs from that of Karshan in that his interpretation of caries-freedom was solely based on mouth mirror and explorer and roentgenographic examination without confirmation by the *L.a.* index determination. In our experience, the *L.a.* index is the most reliable and practical test at the present which indicates caries susceptibility or immunity. By this means the extreme groups (CF and CA) are clearly distinguished from any intermediate groups, such as "arrested-caries" and "miscellaneous" groups (Karshan).

In our former report we stated: "The entire group of 108 individuals had definite caries activity. This was indicated by the finding of new enamel caries as determined by period clinical examinations during the time in which the salivary analyses were made." Karshan feels that "This description leaves the possibility entirely open that Becks' 'caries' group comprised or consisted largely of cases which would have been designated 'miscellaneous' by the present author." It must be emphasized again that when we speak of definite caries-activity, we mean individuals with disintegration of enamel and dentin, and high *L.a.* counts. The CF and CA groups used by us in the previous study were university students, and had been checked every 6 months for *L.a.* index and new cavities. In the selection of our material, we used cases which, over a period of 2 years, had either, 1, no cavities or lactobacilli or, 2, a large number of new cavities and high *L.a.* index.[5] All students were checked by roentgenograms, mouth mirror and explorer within two weeks prior to salivary analyses,

[4] *Lactobacillus acidophilus* will be referred to as "*L.a.*"

[5] Of this total of 198 individuals examined, only 3 in the CF and 6 in the CA groups were exceptions on the basis of the L.a. index findings. Their exclusion from the master charts, however, would not have altered the end results.

and the majority also had *L.a.* index determinations made at the time the saliva was taken for the calcium and phosphorus studies. In those instances, where we were unable to obtain additional samples for bacteriological study on the day of salivary analysis, the last previous count was accepted if it had been made not longer than 2 weeks before. The possibility mentioned by Karshan, that our caries group might not have been caries-active, is therefore entirely invalid.

Differences in technical procedure frequently have led to variations in results as pointed out by Becks and Wainwright (1, 2, 3, 15, 16). The differences between Karshan's (12) and our (7) technical approach consist in: 1. Karshan used saliva collected 1 to 3 hours after breakfast, which he calls resting saliva, while we call resting saliva the one collected *before* breakfast. This might lead to differences in results. 2. While preliminary centrifugation has been used in the past to eliminate food and debris from the salivary sample, Karshan has preferred to omit this step; which again might be responsible for differences in results. However, since the CF and CA groups were handled in the same manner in Karshan's as well as in our studies, it is not likely that a difference due to caries-activity would have been masked. 3. In answering the question, should resting or activated saliva be used for studies of this type, it may be stated that according to our experience, resting saliva is preferable because its composition is less subject to extraneous fluctuations (4, 5). On the other hand, since Karshan has found statistically significant differences in calcium and phosphorus averages, not only in resting but also in activated saliva, it was decided to continue our previous investigation on new material and to use the same technical approach chosen by Karshan, with the inclusion of activated as well as resting saliva.

FURTHER STUDIES OF SALIVARY CALCIUM AND PHOSPHORUS ANALYSES OF CARIES-FREE AND CARIES-ACTIVE INDIVIDUALS (NEW DATA)

A. Selection of Material and Approach

As in the former report, members of the student body of the University of California in Berkeley were used for these new tests.[6] They were segregated on the basis of caries-freedom and caries-activity according to the principles set forth previously. A thorough intra-oral dental examination with mirror and explorer as well as dental roentgenograms and *L.a.* studies were made at the same appointment the samples for calcium and phosphorus studies were taken. The saliva of all CF individuals showed no lactobacilli present on 2 successive days and the saliva of all CA individuals had extremely high counts ranging from 2,000 to 665,000 per cc. of saliva. (All exceptions were eliminated.) The resting and activated saliva samples were taken on the same morning. The individuals were asked to come 1 to 3 hours after breakfast and their cooperation in this respect was closely checked. The resting saliva was taken by the drooling method which was followed by the collection of paraffin activated saliva (1 gram paraffin).

[6] The writers wish to express their sincere gratitude to Dr. William G. Donald, University Physician, and to the Dental Staff of Cowell Memorial Hospital, in particular to Dr. Donald Horner, for their efficient cooperation and assistance which made these studies possible.

Twenty-five individuals in each group were thus studied. Their ages ranged from 17 to 24 and from 17 to 26 in the CA group. The striking similarity of these age ranges is expressed in the means of these 2 groups (Table III) which are identical at 20.20 years. Of the CF, 12 were males and 13 females, and of the CA, 18 were males and 7 females. Karshan's approach (11) was employed and none of the saliva samples were centrifuged.

TABLE I

Chemical analysis of resting and activated saliva of 25 caries-free individuals

ANALYSIS NUMBER	SEX	AGE	RESTING SALIVA					ACTIVATED SALIVA				
			Rate of flow	Calcium		Phosphorus		Rate of flow	Calcium		Phosphorus	
			cc./hr.	*mg. %*	*mg./hr.*	*mg. %*	*mg./hr.*	*cc./hr.*	*mg. %*	*mg./hr.*	*mg. %*	*mg./hr.*
1809	♀	17	19.2	5.1	0.98	12.8	2.46	118.3	5.6	6.62	8.8	10.41
1817	♂	18	20.1	5.7	1.14	14.2	2.85	92.0	5.9	5.43	10.7	9.84
1813	♀	18	24.0	5.6	1.34	18.2	4.37	150.0	5.4	8.10	13.6	20.40
1853	♂	19	12.4	4.6	0.57	16.9	2.10	105.3	4.3	4.53	9.0	9.48
1851	♀	19	9.8	5.1	0.50	23.4	2.29	82.8	5.8	4.80	12.5	10.35
1855	♀	19	11.2	5.0	0.56	16.1	1.80	104.0	5.2	5.41	11.4	11.86
1847	♂	19	15.5	6.0	0.93	23.7	3.67	136.5	5.3	7.23	12.6	17.20
1815	♀	19	11.5	5.1	0.59	19.7	2.26	124.8	5.4	6.74	11.4	14.23
1819	♂	19	9.2	5.7	0.52	17.2	1.58	63.3	6.2	3.92	12.2	7.72
6131	♀	20	6.3	6.3	0.40	20.7	1.30	82.5	5.9	4.87	13.9	11.47
6147	♂	20	21.6	4.8	1.04	13.4	2.89	184.0	5.6	10.30	7.5	13.80
6071	♀	20	26.2	5.8	1.52	17.9	4.69	166.1	4.8	7.97	11.3	18.77
6073	♂	20	34.0	6.1	2.07	15.1	5.13	45.8	5.6	2.56	10.7	4.90
6083	♀	20	7.7	7.5	0.58	25.0	1.92	72.8	6.5	4.73	12.9	9.39
6069	♂	21	10.6	7.0	0.74	18.8	1.99	152.8	5.8	8.86	15.1	23.07
6075	♀	21	36.6	5.4	1.98	18.2	6.66	72.0	5.8	4.18	12.7	9.14
6077	♀	21	14.8	4.5	0.67	17.3	2.56	112.6	4.6	5.18	11.1	12.50
6125	♀	21	15.9	7.0	1.11	27.2	4.32	120.0	6.9	8.28	14.3	17.16
6133	♂	21	12.6	5.9	0.74	12.9	1.62	65.4	7.2	4.71	9.9	6.47
6139	♂	21	20.5	5.6	1.15	28.8	5.90	148.5	6.5	9.65	14.1	20.94
6141	♂	21	13.3	6.1	0.81	21.4	2.85	288.0	6.0	17.28	12.3	35.42
6079	♀	22	8.5	6.4	0.54	23.4	1.99	156.0	6.4	9.98	11.2	17.47
6119	♀	22	20.5	5.8	1.19	12.6	2.58	104.8	5.8	6.08	9.2	9.64
6145	♂	23	50.4	5.5	2.77	20.0	10.08	138.0	6.7	9.25	11.6	16.01
6129	♂	24	14.4	6.4	0.92	12.2	1.76	127.5	5.9	7.52	11.1	14.15

B. Results

The results by individuals of resting as well as activated salivas are given in Tables I and II. They have been arranged for convenience and comparison according to ascending age in the same manner as Tables II and III of our previous report (7).

RESTING SALIVA

Rate of Flow: The ranges of rates of flow of the CF and CA groups are practically identical while the means (M) differ by only 0.52 cc./hr. Since the rate

of salivary flow influences the mg. % values of calcium and phosphorus (4, 5), it is important to note that both groups are practically identical in this respect as well as age.

Calcium: The arithmetic means of calcium mg. % are only 0.24 mg. % higher in the CF than in the CA group. The difference is even less in the mg./hr. values (0.07 mg./hr.).

Phosphorus: The differences for inorganic phosphorus are 2.14 mg. % and 0.48 mg./hr. which are also statistically not significant.

TABLE II

Chemical analysis of resting saliva of 25 individuals with active caries

ANALYSIS NUMBER	SEX	AGE	RESTING SALIVA					ACTIVATED SALIVA				
			Rate of flow	Calcium		Phosphorus		Rate of flow	Calcium		Phosphorus	
			cc./hr.	*mg. %*	*mg./hr.*	*mg. %*	*mg./hr.*	*cc./hr.*	*mg. %*	*mg./hr.*	*mg. %*	*mg./hr.*
1831	♂	17	16.1	5.8	0.93	16.1	2.59	92.6	4.8	4.44	11.5	10.65
1825	♂	18	19.9	6.2	1.23	15.8	3.14	240.0	6.7	16.08	11.3	27.12
1837	♂	18	12.4	4.6	0.57	14.0	1.74	55.5	3.6	2.00	7.5	4.16
1843	♀	18	20.4	5.2	1.06	18.5	3.77	186.0	5.5	10.23	12.0	22.32
1823	♂	19	22.6	5.4	1.22	14.7	3.32	107.0	5.7	6.10	12.9	13.80
1827	♂	19	27.5	4.6	1.26	14.9	4.10	65.2	4.0	2.61	10.9	7.11
1829	♂	19	13.9	6.2	0.86	18.9	2.63	136.0	5.6	7.62	10.8	14.69
1833	♂	19	18.0	5.4	0.97	16.4	2.95	228.0	5.3	12.08	13.5	30.78
1839	♂	19	8.8	6.0	0.53	16.2	1.42	99.0	4.9	4.85	11.7	11.58
1841	♀	19	11.3	5.8	0.66	18.6	2.10	63.7	5.0	3.18	13.2	8.41
6089	♀	20	6.4	5.0	0.32	14.8	0.95	62.0	4.2	2.60	10.5	6.51
6095	♂	20	16.8	4.3	0.72	19.4	3.26	68.0	5.2	3.54	14.2	9.66
6109	♂	20	10.2	6.1	0.62	16.6	1.69	140.0	7.0	9.80	16.2	22.68
6107	♂	20	16.1	5.5	0.88	17.0	2.74	117.3	4.8	5.63	12.0	14.08
6085	♀	21	10.1	6.9	0.70	18.7	1.89	118.5	6.3	7.46	13.4	15.88
6097	♂	21	40.9	4.8	1.96	12.6	5.15	270.0	5.8	15.66	10.9	29.43
6099	♀	21	10.7	5.4	0.58	12.9	1.38	99.0	7.9	7.82	13.5	13.36
6103	♂	21	20.0	6.5	1.30	19.4	3.88	64.7	4.5	2.91	12.0	7.76
6105	♂	21	16.6	5.6	0.93	24.0	3.98	142.3	4.4	6.26	13.9	19.78
6113	♀	21	25.2	4.7	1.18	12.1	3.05	127.5	5.1	6.50	6.8	8.67
6123	♂	21	19.6	6.0	1.18	17.2	3.37	85.8	6.6	5.66	10.1	8.66
6127	♂	21	7.6	5.8	0.44	14.1	1.07	114.0	4.8	5.47	10.9	12.43
6087	♂	22	14.5	4.9	0.71	21.3	3.09	111.0	5.3	5.88	15.2	16.87
6117	♂	24	15.4	6.1	0.94	18.0	2.77	128.0	5.8	7.42	12.7	16.26
6111	♀	26	32.8	5.3	1.74	11.4	3.74	160.5	5.0	8.02	9.2	14.77

ACTIVATED SALIVA

Rate of Flow: As in resting saliva, we find practically identical increases in rate of flow due to activation. The averages of CF and CA groups compare very favorably (120.55 and 123.26 cc./hr., respectively).

Calcium: The mean values for calcium mg. % show a slightly greater difference than those of resting saliva; however, the difference is not statistically significant. The same is true of the calcium mg./hr. values.

Phosphorus: The phosphorus mg. % averages are practically the same (11.64 and 11.87 mg. % resp.), and again the difference between means of the mg./hr. values does not prove to be statistically significant.

C. Comment

All arithmetic means for calcium and phosphorus mg. % and mg./hr. values prove, without exception, not to be significantly different; neither in resting saliva nor in in stimulated saliva. Even though the values of the CF group are slightly higher, with the exception of the phosphorus values of activated saliva, the differences

TABLE III

Statistical evaluation of data in tables I and II

	RANGE		AVERAGE (M)		STANDARD DEVIATION (σ)		SIGNIFICANCE OF DIFFERENCE BETWEEN MEANS ($M_{CF} - M_{CA}$)
	CF*	CA*	CF	CA	CF	CA	
Age	17–24	17–26	20.20	20.20	1.57	1.90	No
Group I: Resting Saliva							
Rate of Flow, cc./hr.	6.3 – 50.4	6.4 – 40.9	17.78	17.35	10.05	7.85	No
Calcium, mg. per cent	4.5 – 7.5	4.3 – 6.9	5.76	5.52	0.74	0.65	No
Calcium, mg./hr.	0.40– 2.77	0.32– 1.96	1.01	0.94	0.56	0.38	No
Inorganic Phosphorus, mg. per cent	12.2 – 28.8	11.4 – 24.0	18.68	16.54	4.57	2.92	No
Inorganic Phosphorus, mg./hr.	1.30– 10.08	0.95– 5.15	3.26	2.79	1.97	1.03	No
Group II: Activated Saliva							
Rate of Flow, cc./hr.	45.8 –288.0	55.5 –270.0	120.55	123.26	48.98	55.90	No
Calcium, mg. per cent	4.3 – 7.2	3.6 – 7.9	5.80	5.35	0.68	0.97	No
Calcium, mg./hr.	2.56– 17.28	2.00– 16.08	6.97	6.79	2.95	3.63	No
Inorganic Phosphorus, mg. per cent	7.5 – 15.1	6.8 – 16.2	11.64	11.87	1.83	2.12	No
Inorganic Phosphorus, mg./hr.	4.90– 35.42	4.16– 30.78	14.07	14.70	6.35	7.05	No

* CF = Caries-free.
CA = Caries-active.

can by no means be related to the dental caries process, because they fall entirely within the influences of chance variation, and especially because of the small number of 25 in each group.

In comparing the resting saliva results obtained in this study with those previously reported by us (7), very slight differences are noted.[7] While the inorganic phosphorus values are higher in the present study, the calcium values are practically the same. The difference in phosphorus values may be due in part to the fact that there were a number of rate of flow values below 11.1 cc./hr. in the present study, while these were not included in Table VII of the former report,

[7] Table VII (7) is used for comparison because of the more similar age distribution.

and in part to the fact that two different types of saliva were used. In the former study, resting saliva was collected before breakfast, while in this study samples were taken after breakfast when the salivary gland was in a different physiological state. Since these samples were collected after breakfast, it must be recalled that activation by different stimuli may produce variable calcium and phosphorus values depending entirely upon the selective response of the salivary glands, even to the extent of increasing the calcium concentration by activation (6). On the other hand, since fluctuations in salivary phosphorus content can occur so readily during activation and since the saliva was collected after breakfast, it is quite evident that for a comparative study of CF and CA groups at present only saliva can be used which is uninfluenced by various types and intensities of stimulation. The writers question the value of any experimental data based on saliva samples collected after a meal, with or without additional activation by paraffin. The closeness of the arithmetic means of calcium mg. % of resting saliva and phosphorus mg. % in activated saliva must be considered accidental, especially in view of the fact that groups of only twenty-five cases were studied.

In comparing the present results for activated saliva with those of Karshan, Krasnow and Krejci (13), Krasnow (14), Hubbell and Bunting (9), Hubbell (8), and White and Bunting (17), a definite similarity can be seen. The arithmetic means of the CF patients are slightly higher: however, the important facts remain that the differences are not statistically significant and that CF individuals show values that are just as low as any of the CA group. Karshan is one of the few investigators who has found a significant difference not only in resting saliva, but also in stimulated saliva. The writers are, on the strength of the present findings and those of their previous report, unable to confirm Karshan's findings with regard either to resting or activated saliva.

SUMMARY AND CONCLUSIONS

A study was undertaken of the total calcium and inorganic phosphorus composition of salivary samples collected 1 to 3 hours after breakfast, with and without paraffin activation, and in comparable groups of caries-free and caries-active patients. The purpose of this study is to check further the claims of Karshan that statistically significant differences exist between these groups due to caries activity. In former studies in this laboratory, only resting saliva samples, taken before breakfast and before brushing the teeth, were used. However, in view of the fact that Karshan considers that differences in concept of clinical criteria for caries-activity and freedom, time of collection and type (resting or activated) of samples, and preliminary treatment make comparison of our previous studies impossible, these were extended and all above differences in approach were eliminated and the exact technique of Karshan was followed. The results can be summarized as follows:

The difference between arithmetic means of all calcium and phosphorus mg. % and mg./hr. values of resting as well as activated saliva of 25 caries-free and 25 caries-active individuals fall within the same range and are not significantly different according to statistical evaluation.

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VARIATIONS IN THE pH OF SALIVA OF FIVE INDIVIDUALS[1]

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When searching the literature concerning the hydrogen ion concentration of saliva, one finds a great range of averages expressed in terms of pH and of methods used for its determination such as indicator paper, colorimetric and electrometric methods. The literature is very limited in referring to the use of the glass electrode to determine the pH of saliva. In Black's "Operative Dentistry" (1) is the statement that up to 1936 no extensive reports as to the pH of saliva by means of the glass electrode were available. Wessinger (2) determined the pH of saliva by means of the glass electrode and compared the readings with those obtained by the quinhydrone electrode, but no averages or statistics were made as he was interested only in comparisons. Also, 3 cases were listed by Wessinger in which 3 or 4 tests were made upon each individual, but no pH averages were given.

Brawley (3) determined 6.75 as an average pH of 3404 cases, using the colorimetric method. He found that readings taken immediately after collections did not vary so appreciably as to warrant collecting saliva under oil, as suggested by Starr (4) and others. Grossman and Brickman (5) reported that averages determined by earlier investigators varied greatly. Their own average (diurnal) was pH 6.7 and was determined with the quinhydrone electrode. No statistical analysis was made. These men reported, also, that while the pH level of saliva varied with different individuals, it was fairly constant in the same person. They tested 3 individuals twice at a 10 month interval. While comparing colorimetrically the pH of saliva with the incidence of caries, Swerdlove (6) gave 6.69 as the average pH of saliva of 351 people. His data were treated statistically.

Although investigators have tested the pH of saliva of thousands of individuals, data pertaining to changes in the pH of saliva during the day are not numerous, and no reference was found dealing with repeated tests during the day upon a few individuals. The early work of Bunzell in 1923 (7) dealt in part with colorimetric determinations of several individuals made irregularly in the morning and in the afternoon for a few scattered days. Bunzell stated that he found no difference in the pH of saliva in the morning and afternoon.

Grossman and Brickman (8) emphasized that while the salivary pH of individuals varied hourly and daily, it was fairly constant for each individual. A drop in pH occurred in saliva after eating, but returned to the original pH within an hour. These same men (5) reported no apparent drop in the pH of subjects who remained awake for 24 hours, but a definite nocturnal drop occurred if the

[1] Received for publication January 9, 1943. Revised by author March 19, 1943.

subjects slept and were awakened only long enough to collect a saliva sample. The latter subjects showed definite increases in pH about 8 or 9 o'clock in the morning. An increase in pH of saliva in the afternoon over the morning was noted by Krasnow (9) but no definite conclusion was presented. Brawley (3) pointed out the need of studying factors which cause changes in the hydrogen-ion concentration of saliva such as diurnal influences, age and sex. He stressed the latter two. Saunders (10) compared the pH of saliva in epileptics and non-epileptic individuals at 15 minute intervals for 5 to 7 hours and found slight changes in the non-epileptics, but marked changes in epileptics.

The purpose of this study was to test repeatedly with the glass electrode the pH of saliva of a few individuals to determine (a) the average of all readings taken, and to find if this average is similar to those reported in the literature; (b) the mean for each person and its statistical significance; (c) the extent in which the saliva of these individuals collectively fluctuated in pH during the day; and (d) the variation in pH of each subject's saliva during the day from the group average, and also, from each one's own mean. No attempt has been made to completely cover the literature as the papers cited provide excellent references to the earlier work in this subject.

METHODS

Determinations of pH were made with the glass electrode using a pH electrometer. Normal unstimulated (resting) saliva was collected in the mouth until 5 or 6 ml. accumulated before spitting into the sample tube. Readings were made in less than 1 minute after expectoration so that it was unnecessary to cover the samples with paraffin oil. Comparisons were made regarding this point and it was found that no noticeable changes occurred within the short time interval mentioned; this is in agreement with Starr (4). The 5 subjects in the experiments were known by the letters S., C., P., E. and H.

Readings were taken daily at 9 and 11 o'clock in the morning and at 1, 3 and 5 o'clock in the afternoon. The tests were made on consecutive days during the last week of October followed by 2 broken periods during the first and second week of November, 1942. The compiled data were analyzed statistically to decide whether or not the results were of significance. The procedure was that advocated by Snedecor (11).

EXPERIMENTAL DATA

A total of 276 pH determinations were made upon the resting or unstimulated saliva of 5 staff members of the School of Dentistry, University of Kansas City. The average[2] pH was 6.72 $\mp$ 0.015 (i.e., standard error). This is in close agreement with recent investigators who used colorimetric methods.

Forty-six tests were made upon the saliva of S.; his mean pH was 6.78. Table I shows this figure to be statistically insignificant from the average of 6.72 because the group mean ratio was 1.60. This table also shows the saliva of C., (mean pH 6.59) to be significantly more acid than the average; P.'s saliva was

[2] The term "average" connotes "mean" and the 2 terms are used synonymously.

average and not significant; E.'s saliva was 6.66, which was significantly more acid than the average; and the saliva of H. was significantly less acid (6.84) than the general average of 6.72. Therefore, by testing the saliva of a few individuals many times, (each person was tested from 46 to 74 times) it was shown that the average pH agreed with Brawley (3), Grossman and Brickman (5), and Swerdlove (6), who usually made one determination on a single individual. Although the total number of readings gave an average which agreed with these investigators, it is pointed out that significant deviations occurred in 3 of the 5 individuals.

Fig. 1 shows the frequency distribution curve of 276 pH saliva values of the 5 individuals tested, and the frequency distributions for each person. Note that they all tend to follow the normal distribution curve, and that the pH range is from 5.7 to 7.5.

It was desirable to know whether the variations of the pH averages of saliva among the subjects were important, or whether each subject varied so much in his single pH readings as to cause the variations to have little value. Therefore,

TABLE I

pH of saliva using the glass electrode

	S	C	P	E	H	TOTAL
No. readings	46	46	50	74	60	276
Average pH	6.78	6.59	6.71	6.66	6.84	6.72
Std. deviation	±0.2545	±0.2875	±0.2250	±0.1683	±0.2134	±0.2420
Std. error of mean	∓0.0375	∓0.0424	∓0.0318	∓0.0196	∓0.0276	∓0.0150
Total mean ratio	1.60	3.07*	0.314	3.06*	4.35*	
1% significance	2.69	2.69	2.67	2.65	2.66	

* Significantly different from 6.72 average.

an analysis of variance was made, and the summary of the data is presented in Table II. The F ratio of 9.68 indicates a high significance of variation of the means among the subjects. Such a figure leaves little doubt of the homogeneity of the mean pH saliva value of each person, and there is a minimum probability that the differences were due to aberrant single tests. Even a ratio of 3.39 would be considered significant as it indicates that there was only one chance out of 100 tests of the variations being due to single readings of the same individual. Therefore, it can be safely said that C. and E. had salivas which were definitely acid, and H. definitely less acid that the average without fear of heterogenous single readings giving rise to erroneous mean values. Also, the means of S. and P. were actually true values lying near the mean 6.72.

Two hundred fifty-two pH determinations made upon saliva of the 5 subjects were analyzed for bi-hourly fluctuations. Twenty-four pH readings of E. were not included in the bi-hourly study because they were made at hours other than those used. However, the mean for E. did not change. Table III shows the average pH of saliva for the hours of 9 and 11 o'clock in the morning and 1, 3 and 5 o'clock in the afternoon. Analysis of the variance gave the highly significant

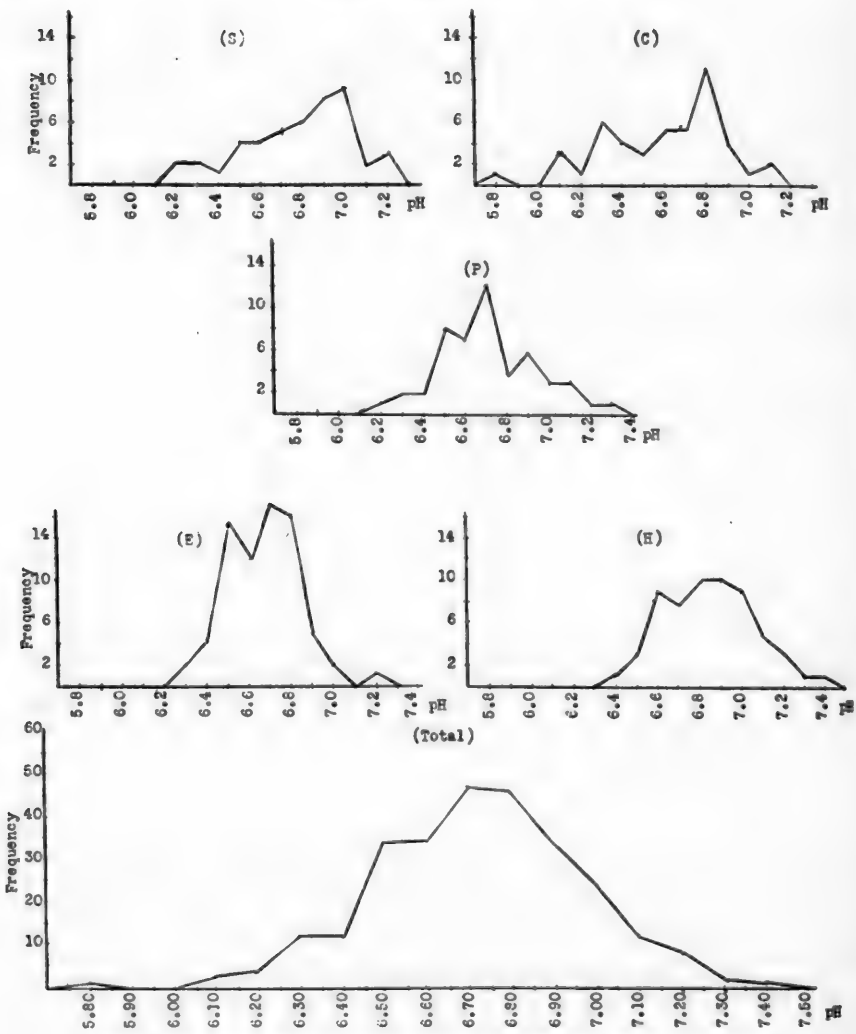

FIG. 1. Frequency curves for total and individual pH determinations

TABLE II

Analysis of variance of the data given in Table I

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE
Total	275	16.271173	
Between group means	4	2.034495	0.508624
Between readings of same individual	271	14.236678	0.052534

F = 0.508624/0.052534 = 9.68—highly significant.
1% Significance = 3.39.

ratio of 4.9 for variations of the bi-hourly means; therefore, chance did not resolve these figures into meaningless numbers, and importance was attached to the variations during the day for these periods. The mean saliva pH of 6.60 at 9 A.M. was followed by a sharp rise, which later dropped during the afternoon, but by 5 P.M. the saliva mean was at a higher pH than at any time during the day. The dotted line in *fig. 2* gives a better conception of the changes in the pH of saliva throughout the day. In addition to an analysis of variance in Table III, each period was tested for a significant difference from the average of 6.72 established for the entire group. The mean pH of 6.60 for saliva at 9 A.M. was significantly less than the over-all average of 6.72 because the ratio was 3.02

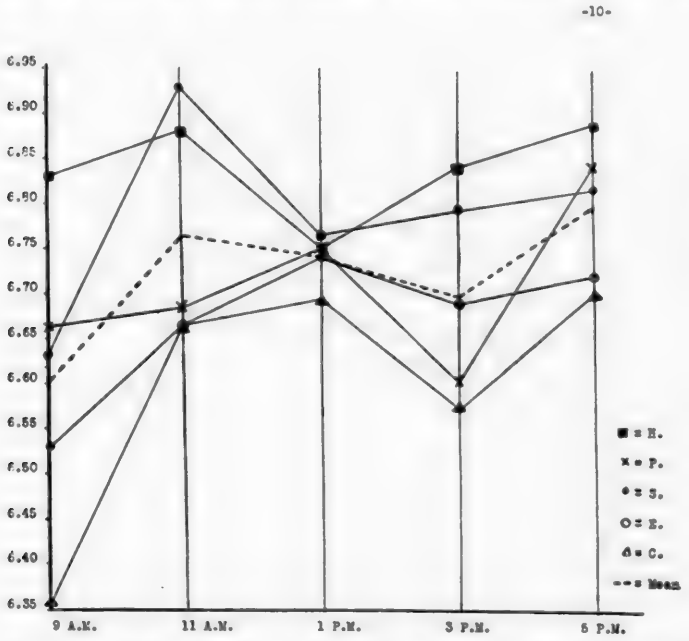

FIG. 2. The mean pH of saliva during the day for each subject and the mean for all

whereas only 2.67 was required for 1% significance. At 5 P.M. the mean pH was 6.79 which probably was significant because the ratio was midway between the values required for 1% and 5% significance. The intervening data were not significantly different from 6.72. The results, which covered a period of 10 to 12 days, suggested the early morning and late afternoon as periods of considerable differences in the hydrogen-ion concentration of saliva from the over-all mean, and during the mid-day they deviated only slightly.

The remaining data were based upon individual variations during the day. Variance among the subjects was analyzed statistically for individual salivary pH averages at the previously specified hours (read down in Table IV). The 9 A.M. means had a highly significant F value of 5.19, therefore, the pH of saliva of subjects were statistically at variance from one another for this time period.

Apparently chance did not account for the low pH mean of 6.36 for C. and the high pH mean of 6.83 for H. Unknown factors must have played important roles to cause these variations. At 11 A.M. the F value determined by an analysis of variance indicated probable significance because it was only slightly below the value for 1% significance, but definitely above the 5% point of significance. Thereafter the F values indicated no significant variance of the means. A minimum difference was noted at 1 P.M. At this time C., who diverged the greatest amount, ate after 1 o'clock; the other subjects ate at 12 noon. It seems possible that eating accounted in part for this great similarity in pH of saliva. In general, the salivas began to lose their subject identities as the day progressed

TABLE III

Summarized bi-hourly variations in the pH of saliva of 5 individuals

	9 A.M.	11 A.M.	1 P.M.	3 P.M.	5 P.M.	TOTAL
No. readings	53	51	50	50	48	252
Mean pH	6.60	6.76	6.74	6.70	6.79	6.72
Std. deviation	±0.290	±0.2282	±0.2082	±0.2431	±0.2197	±0.242
Std. error of mean	∓0.0398	∓0.0320	∓0.0294	∓0.0344	∓0.0317	∓0.015
Total mean ratio	3.02*	1.25	0.70	0.58	2.21*?	
1% significance	2.674	2.678	2.680	2.680	2.686	

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE
Total	251	15.20702	
Between hourly means	4	1.11670	0.279175
Between readings of same hour	247	14.09032	0.057050

F = 0.279175/0.057050 = 4.9—highly significant.

1% Significance = 2.4.

* Statistically significant.

*? Probably significant.

and tended toward a homogeneity of all subjects. This was especially marked at noon.

By reading across Table IV, the individual bi-hourly salivary pH means are observed, and an analysis of variance is summarized for each subject at the extreme right of the table. No one varied significantly during the day. For example, the F value for S. was 1.21, but to be significant needed to be 13.74. C. approached significance, but probably should not be considered at variance during the day. This analysis leaves little doubt that each individual tested did not vary greatly during the day when following the usual daily working routine, and it adds importance to the data in Table III and *fig. 2*.

Further reference is made to *fig. 2*. The graph for each subject follows closely the average pH bi-hourly graph. At 9 A.M. the pH of saliva of the 5 subjects varied significantly from one another. By 11 A.M. all salivas were noticeably less acid than at the earlier period and the dissimilarity among individuals was

TABLE IV

Mean pH of saliva of 5 individuals at bi-hourly intervals. Analysis of variance of individual variations for each period and for the day. Also, individual deviation from the bi-hourly mean and from the daily mean

	9 A.M.	11 A.M.	1 P.M.	3 P.M.	5 P.M.	DAILY	ANALYSIS OF VARIANCE FOR INDIVIDUAL DURING DAY	
							F value	F required for 1% sig.
(S.)								
Mean pH	6.63	6.93	6.76	6.79	6.81	6.78	1.21	13.74
Std. error of mean	∓0.089	∓0.054	∓0.094	∓0.059	∓0.102	∓0.038		
Ratio with daily mean	1.69	2.76	0.07	0.17	0.30			
Ratio with bi-hourly mean	0.34	3.13	0.21	1.52	0.20			
1% significance	3.17	3.36	3.36	3.25	3.71			
(C.)								
Mean pH	6.36	6.66	6.69	6.57	6.70	6.59	2.67	3.82
Std. error of mean	∓0.115	∓0.074	∓0.045	∓0.113	∓0.079	∓0.042		
Ratio with daily mean	2.01	0.94	2.24	0.18	1.39			
Ratio with bi-hourly mean	2.10	1.34	1.12	1.15	1.14			
1% significance	3.25	3.25	3.36	3.25	3.71			
(P.)								
Mean pH	6.66	6.68	6.75	6.60	6.84	6.71	1.66	3.77
Std. error of mean	∓0.055	∓0.074	∓0.062	∓0.079	∓0.061	∓0.032		
Ratio with daily mean	0.90	0.40	0.65	1.39	2.03			
Ratio with bi-hourly mean	1.09	1.08	0.16	1.27	0.78			
1% significance	3.25	3.25	3.25	3.25	3.25			
(E.)								
Mean pH	6.53	6.66	6.74	6.68	6.72	6.66	2.50	3.77
Std. error of mean	∓0.040	∓0.043	∓0.059	∓0.063	∓0.042	∓0.020		
Ratio with daily mean	3.29*	0.00	1.36	0.32	1.42			
Ratio with bi-hourly mean	1.79	2.33	0.00	0.32	1.65			
1% significance	3.25	3.25	3.25	3.25	3.25			
(H.)								
Mean pH	6.83	6.88	6.75	6.84	6.89	6.84	1.03	3.68
Std. error of mean	∓0.067	∓0.049	∓0.073	∓0.054	∓0.065	∓0.028		
Ratio with daily mean	0.15	0.81	1.24	0.00	0.77			
Ratio with bi-hourly mean	3.45*	2.43	0.14	2.60	1.53			
1% significance	3.11	3.11	3.11	3.11	3.11			
Bi-hourly mean	6.60	6.76	6.74	6.70	6.79			
Analysis of variance for bi-hourly periods								
F value	5.19*	3.40*?	5.98	2.55	1.44			
F required for 1% significance	3.74	3.76	13.72	3.77	3.79			

* = Statistically significant.

*? = Probably significant.

probably statistically at variance. After the close similarity in pH of saliva at 1 P.M., divergence again occurred but not significantly. Two subjects were less acid at 3 P.M. than at 1 P.M., and all subjects became less acid at 5 than at 3 o'clock. From a statistical standpoint importance can be placed only upon the 9 A.M. and 11 A.M. periods.

Two additional points should be mentioned. First, question arose as to whether or not the saliva of any one subject was significantly different during the day from his own mean. The mean, the standard error, ratio and 1% significance are given in Table IV for each time period, and the former is compared with the daily mean pH of each subject. No one had a bi-hourly mean which was significantly different from his daily mean, except E. at 9 A.M. Therefore, it may be said that a subject did not ordinarily deviate at bi-hourly periods from his daily mean to a point of significance during the day.

Another question pertained to whether or not each individual bi-hourly mean differed from the total bi-hourly mean of all subjects. (In Table IV, the second ratio given refers to the bi-hourly mean.) Statistically the deviations were not significant except H. at 9 A.M. who had a salivary mean pH of 6.83 as compared with 6.60 for the total bi-hourly mean. S. at 11 A.M. had a mean pH which approached significance. Upon this basis it may be stated that for the most part these subjects did not deviate significantly from the mean pH at any one period. Both of these points add importance to the data of Table III and *fig. 2*.

DISCUSSION

Although great variations have been reported for the average pH of saliva, better methods now available are gradually restricting the variability range. The most accurate method for determining the hydrogen-ion concentration is generally conceded to be the glass electrode. There is general agreement now that saliva is normally slightly acid. Since the figures herein reported were treated statistically, it is felt that they substantiate the growing belief of recent investigators that the true average pH of saliva is near 6.75. The procedure of using a few individuals who were tested repeatedly throughout the day from 10 to 12 days is in contrast to the majority of reports based upon single tests of many individuals.

Dental caries was not considered in this report, but mention should be made that all subjects were examined in the clinic and found to have from 2 to 8 carious teeth. H. had the highest pH and 8 cavities; C. had the lowest pH and 2 cavities. Of course this is of no statistical significance.

Six additional points were considered in the statistical analysis of the experimental data in this paper; (a) analysis of variance of the means derived from all subjects for the 5 time periods; (b) analysis of variance of means for each subject for the 5 time periods; (c) analysis of variance of the 5 subjects for each time interval; (d) the deviation of each bi-hourly mean from the daily mean of all subjects; (e) the deviation of the individual's bi-hourly means from his daily mean; and (f) the deviation of individual's bi-hourly means from the mean for each bi-hourly period.

The fact that point (a) was statistically significant proves that considerable variations occurred during the working hours of the day. The low point in the early morning is in agreement with Grossman and Brickman (5). Perhaps these salivary changes during the day were influenced directly by the body metabolism and the greater emphasis for individual variations had best be placed upon the early morning rather than the remainder of the day.

Eating may have caused a great similarity of salivary pH for a short time afterwards as observed by the striking resemblence of the individual mean pH at 1 P.M. The individual who deviated greatest did not eat until after the 1 P.M. readings were obtained. The influence of food was lost before 3 o'clock. Grossman and Brickman (5) reported a change in pH following a meal and its disappearance within an hour. Two opposing ideas suggest an explanation for the great similarity of pH following a meal. One is that food may have a stabilizing effect upon the pH of all saliva which causes the disappearance of individual characteristics observed during mid-morning and mid-afternoon. The opposite is plausible, too. Eating may clean the mouth of bacteria, desquamated epithelia, etc., so that the average pH of the saliva would be more likely observed after eating than during the mid-morning or mid-afternoon when these factors would be most active, and hence, a more accurate average pH of saliva could be obtained than at other times of the day.

CONCLUSION

The average pH of saliva of 5 subjects, as determined repeatedly by the glass electrode, was 6.72, however, the subjects varied significantly from one another. The group exhibited significant variations in the pH of saliva during the day, especially during the early morning. The saliva of the group was significantly more acid in the morning and perhaps significantly less acid in the late afternoon than the average for the entire day.

For each time period during the day subjects were compared with one another and were found to vary significantly in the morning. But immediately following the mid-day lunch they were nearly identical. These points suggest a relationship with body metabolism.

During the day the pH of saliva for each subject was not generally different from each one's daily mean or bi-hourly mean, thereby adding importance to the group results.

SUMMARY

The mean pH of saliva tested 276 times in 5 adults was 6.72 ∓ 0.015, which was similar to other published reports. This reading was obtained by using the glass electrode.

An analysis of variance indicated that each individual's average pH of saliva was significantly variable from one another.

Of these averages 2 were significantly more acid than the average 6.72, 2 were not significantly different from the average and one was significantly less acid than the average.

Two hundred fifty-two determinations of the pH of saliva from 5 individuals were analyzed statistically for variations during the day. The readings were taken at 9 and 11 A.M., and at 1, 3 and 5 P.M.

The group of subjects was significantly at variance during the day.

The group of subjects was significantly different from the daily mean at 9 A.M. and probably at 5 P.M., but not at the other periods.

No subject was significantly at variance during the day.

Subjects were significantly at variance with one another at 9 A.M. and probably at 11 A.M., but thereafter were not. That is, as the day progressed individual identities of saliva were lost and tended toward homogeneity.

Subjects did not deviate significantly (1 exception) from their respective individual daily means for each of the 5 periods of time.

Subjects did not deviate significantly (1 exception) from their respective bihourly means for each of the 5 periods of time.

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ANALYSIS OF ORAL REACTIONS TO DILANTIN-SODIUM[1]

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The more widespread use of dilantin-sodium in the treatment of convulsive disorders has stimulated a demand for dental consultations to interpret the significance of the oral reactions encountered. That dilantin often produces changes in the gingival tissues is unchallenged, but no uniformity of opinion exists concerning their incidence, categories, or the features that distinguish them from other abnormalities of the gingivae. This report, based on a two year study of 62 patients in the Neurologic Clinic of the Mount Sinai Hospital, and on an analysis of the literature, is intended to assist the dental clinician by clarifying these points. A working classification of the lesions produced by dilantin is offered, and the essentials of differential diagnosis, the role of aggravating factors and the indicated scope of treatment are discussed.

When, in 1938, Merritt and Putnam (1) introduced dilantin-sodium for the treatment of epilepsy, certain toxic reactions of the drug were already recognized, which resembled those following the administration of other barbiturates. Kimball and Horan (2) divided these manifestations into (a) acute toxic reactions, notably cutaneous rashes and evidences of damage to the nervous system, and (b) late reactions due to chronic irritation, of which the most prominent were gingival hypertrophy, and recurrent gastritis.

The incidence of gingival changes as reported by several investigators shows a striking disparity (Table I), readily explained by two persisting variables. Statistics were compiled largely by internists rather than stomatologists, and therefore finely shaded differences in the gingival picture may easily have been overlooked. Secondly, observations in no instance were based on patients selected according to the period of time covered by the administration of dilantin. Yet in our studies, the percentage of gingival alterations noted, in fact, the entire clinical picture, often changed after prolonged dosage. Many patients were observed who showed little alteration after one year, but developed marked hypertrophy during the second year of treatment. Carrying this point to a legitimate conclusion, inasmuch as the maximum period that any patient has received dilantin does not yet exceed four years, it is probable that changes in greater degree will be noted in the future.

CLASSIFICATION OF GINGIVAL CHANGES

Among the 62 patients examined, 4 were edentulous and exhibited no lesions; another 8 were rejected as unsuitable for statistical inclusion because of frequent interruption of medication. Studies were continued on the remaining 50 pa-

[1] Received for publication November 16, 1942.

tients who were under dilantin therapy for periods extending from 7 months to 2 years, with an average of 23 months. Their daily dosage varied from gr. 1½ to gr. 7½ (0.1 gm. to 0.5 gm.), with an average of gr. 4½ (0.3 gm.). Kimball (3) graded the extent of alterations in the gingivae as 1, 2 and 3 plus, in order of increasing severity. However, in the interest of greater descriptive clarity,

TABLE I ✓

The percentage of gingival reactions in patients receiving dilantin

INVESTIGATOR	NO. OF CASES	PER CENT SHOWING CHANGES
Kimball (3)	152	57
Merritt and Putnam (4)	332	3
Fetterman (5)	28	25
Frankel (6)	48	62
Glickman and Lewitus (7)	55	18
Average		24
The authors	50	52

TABLE II

Classification of patients receiving dilantin-sodium

CLASS	PER CENT OF PATIENTS	DESCRIPTION
O	48	No changes
I	14	Qualitative changes: Increased density as exhibited by marked stippling and granular effect
II	26	Quantitative changes: Increase of tissue limited to: (a) Gingival margin (rolled effect) (b) Interdental papilla (triangled effect)
III	10	Increase of tissue to a point where there is encroachment on the clinical crown
IV	2	Increase of tissue to a point where there is interference with function

additional specific gingival characteristics and recognizable dental landmarks were employed in our classification.

The earliest recognizable signs of reaction to dilantin were so minute as to deserve a designation of qualitative, rather than quantitative alterations. These consisted of increased stippling, and a granular, warty appearance of the gingivo-mucosal surface (Table II). In histologic section this represented a concentration of collagenous material and thickening of the surface epithelium, a picture of

low grade inflammation with extensive round cell infiltration, rather than one of edema or capillary engorgement.

This incipient stage was succeeded by alterations of a distinctly massive nature which assumed either of two forms. One began as a spreading of the sides of the gingival papillae to produce triangulations of the interproximal mucosae, and then lobulation, with cleft formation at the coalescence of the lobules. The second form started as a marginal festoon or crescentic swelling which slowly increased in size, forming a gradual downward roll of tissue upon the crown of the tooth. One patient, as two of us already reported, (8) developed a giant gingival hypertrophy of a pseudo-neoplastic character, which extended so far on the crowns of the teeth as to embarrass mastication (*fig. 3a*).

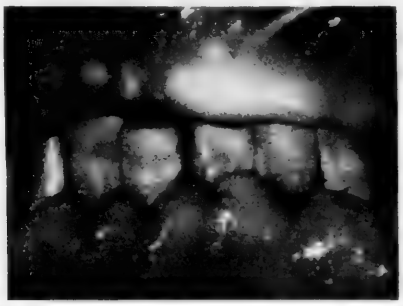

FIG. 1. Gingival hypertrophy of acute lymphatic leukemia which might be overlooked in presence of coincident dilantin therapy.

Fifty-two percent of the patients showed gingival alteration. In only 10 percent, however, was hypertrophy great enough to encroach on the clinical crown, and in only one case was there interference with function. *It was evident that the average reaction to dilantin was mild; the severe reaction was exceptional.*

DIFFERENTIAL DIAGNOSIS

In their superficial appearance, many generalized hypertrophies of the gingivae associated with other causes may simulate the hyperplasia produced by dilantin. Furthermore, in the presence of chronic hypertrophic gingivitis, a history of exposure to dilantin, while presumptive, does not necessarily establish the differential diagnosis. Under such coincidental circumstances a background of leukemia (*fig. 1*) or of endocrine dysfunction may easily be overlooked because of clinical resemblances. As in all conditions simulating neoplasia, systemic inquiry and biopsy are mandatory to establish finally the nature of gingival overgrowth.

FACTORS AGGRAVATING THE HYPERPLASIA OF DILANTIN

The patients in this group were worked up from every conceivable angle in an effort to establish predisposing, aggravating, or etiologic factors. As the figures in Table III indicate, no correlation was demonstrable in connection with sex, age, status of oral hygiene, malocclusions or the therapeutic effectiveness of the drug.

Larger daily dosage did not appear to be consistently associated with substantial overgrowth of the gingival tissues. A longer period of medication however, seemed to be attended by a larger incidence and greater degree of gingival hyperplasia. The more advanced cases, for example, exhibited themselves in patients whose average period of exposure to dilantin was 38 months, compared to an average of 17 months in those with minimal changes. Nevertheless, these associations are not sufficiently consistent entirely to preclude the pos-

TABLE III

Statistical analysis of possible correlation with dilantin

FACTOR	INDEX	NO CHANGES	CHANGES				CONCLUSIONS
		Class O	Classes I-IV	Class I	Class II	Class III	Class IV	
Sex	Male	13	13	5	5	3	0	No correlation
	Female	11	12	2	7	2	1	
Age	Average age in years	29	25	31	23	26	26	No correlation
Dosage	Average Number of Capsules Daily*	2.5	3.2	3.1	3.1	3.0	4.0	+−**
Duration of Dosage	In Months	21.7	24.4	17.5 →	21 →	38.8	33	Probable Correlation
Hygiene	1 = good 2 = fair 3 = poor	1.7	1.7	1.6	1.6	2.0	3.0	No correlation
Effect of Drug on Attacks	1 = optimum 2 = partial 3 = no effect	1.5	1.9	1.7	2.0	2.0	2.0	No correlation
Occlusion	Normal	15	19	6	9	4	0	No correlation
	Trauma	5	3	1	2	0	1	

* Each capsule contains 1½ grains.

** Correlation is within the limits of possible statistical error.

sibility of statistical error, and further studies on larger groups of patient must be awaited before final conclusions are drawn.

As the chronic hypertrophic gingivitis associated with mouth breathing presents points of resemblance to the changes produced by dilantin, the salivary output of these patients was briefly studied. Stimulatory response to the chewing of paraffin occurred within normal limits. Dryness of the mouth was uncommon, nor could any correlated diminution of salivary output be demonstrated.

A control series of 50 patients receiving phenobarbital were examined in the Neurological Clinic. Those receiving phenobarbital alone showed no oral changes, while those in the original group of patients, who received the drug in conjunction with dilantin, displayed no retardation or acceleration of gingival response to the dilantin.

The nutritional status of our patients was investigated in the Medical Clinic with the cooperation of Drs. Herbert Pollack and Max Ellenberg. Careful clinical evaluation of the diet failed to suggest any element of inadequacy to account for the hypertrophy. Seven patients showing the most advanced gingival changes responded within normal limits to tolerance tests for Vitamin B_1, as measured by urinary excretion.

With one exception, roentgenographic studies exhibited no changes in the alveolar bone which could be attributed to or correlated with the hyperplasia (*fig. 3a*). Poor oral hygiene, traumatogenic occlusion, caries and other agencies which impair local resistance, appeared to operate as aggravating, rather than predisposing factors on an already established hypertrophic trend (*fig. 2*). That this trend is present in some patients and not in others is amply demonstrated by the normal gingival configuration found in many of the most neglected mouths. Our present studies continue to be directed toward disclosing the factors which control this tendency.

OTHER ORAL CHANGES FROM DILANTIN

Exceptionally, there may be other sites of reaction to dilantin than the gingival mucosa. Such a case occurred in our series and is briefly presented for the record:

S. B., Hospital No. 34–2175, a white male of 47 years was admitted to the Clinic in 1937 with a diagnosis of idiopathic epilepsy. When his mouth was inspected he had received dilantin for 34 months, with an average daily dose of 4½ grs. (0.3 Gm.). Although the patient was dentulous there was no gingival hypertrophy. However, to the right of the midline of the hard palate, and opposite the premolars, two parallel pairs of small firm nodules were discovered which clinically resembled papillomata. Prompt recurrence followed excision of the largest of these, which histologically showed "striking epithelial hyperplasia, with acanthosis and chronic inflammatory reaction." This picture coincided with those observed in the specimens of gingival hypertrophy and was interpreted as an atypically located oral reaction to dilantin.

TREATMENT

There seemed to be no subjective symptoms associated with the overgrowth of gingival tissue. Most patients were unaware of the process in its early stages, and complained only of disfigurement in the advanced stages. In our opinion, the mildness of the average reaction failed to justify the psychic trauma which must necessarily be inflicted by pointing out this additional physical defect to the already disturbed epileptic patient. For this reason, treatment was instituted only in severe cases or where the patient himself first called attention to the hypertrophy.

Three methods of treatment were employed: (a) Withdrawal of the drug, (b) surgical removal of tissue, and (c) conservative periodontal treatment.

Except where it marked the onset of other and more disturbing toxic reaction the presence of gingival hyperplasia appeared insufficient reason to discontinue

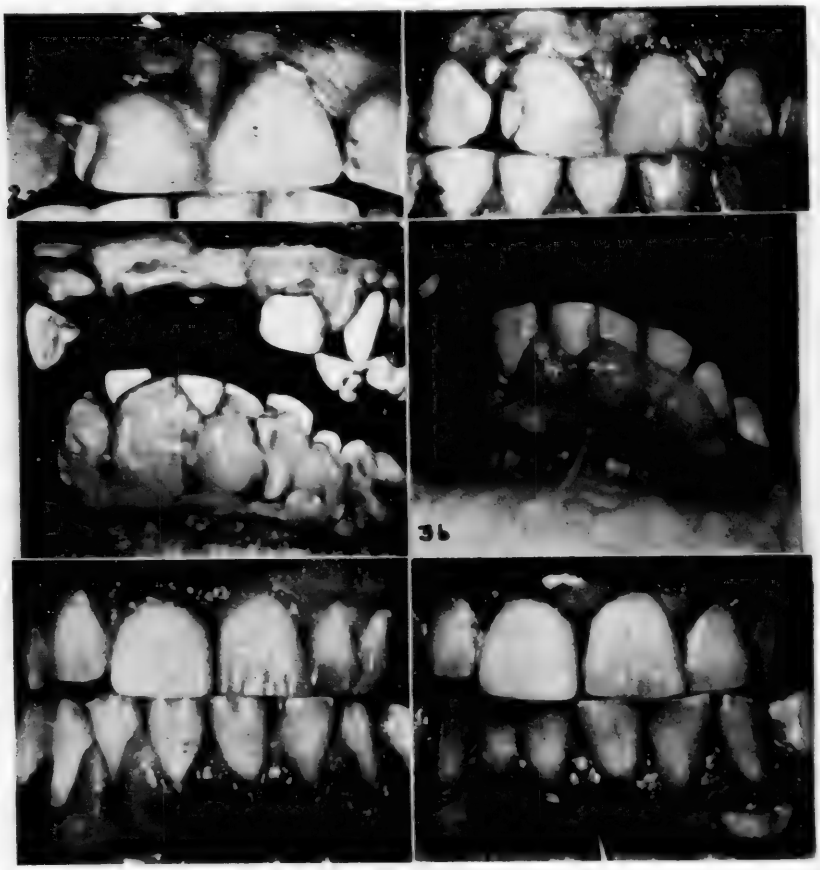

FIG. 2. Aggravating effect of local irritation in dilantin hyperplasia. (a) Patient H. M. (37–3555) Porto Rican female, age 24. Dilantin for 26 months. Average dose 4½ grains daily. (b) One year after discontinuance of dilantin. No treatment instituted. Note withdrawal of enlarged papilla approximating faulty synthetic fillings.

FIG. 3. Recession of tissue following withdrawal of dilantin. (a) M. R. (33–20373) Negress, age 28. Receiving dilantin 36 months. Average dose 6 grains daily. (b) Six months after stopping dilantin. Patient has been maintained on luminal during this interval.

FIG. 4. Effect of conservative treatment. (a) I. M. (40–2579) white male, age 25. Receiving dilantin for 54 months. Average dosage 4½ grains daily. Hypertrophy class III. (b) Three weeks after curetting and instituting home care. Note improvement in lower anterior region.

dilantin. However, in those patients who could be maintained successfully on luminal, a gradual but incomplete resolution of the process occurred (*fig. 3*).

Gingivectomy proved rapid and effective, but unless the patient cooperated

by home treatment and follow-up, rapid recurrence followed. It is recommended only for selected, advanced cases.

Most patients were satisfactorily controlled by orthodox periodontal treatment, augmented by intensive interdental stimulation (*figs. 4, 5*). Salivary

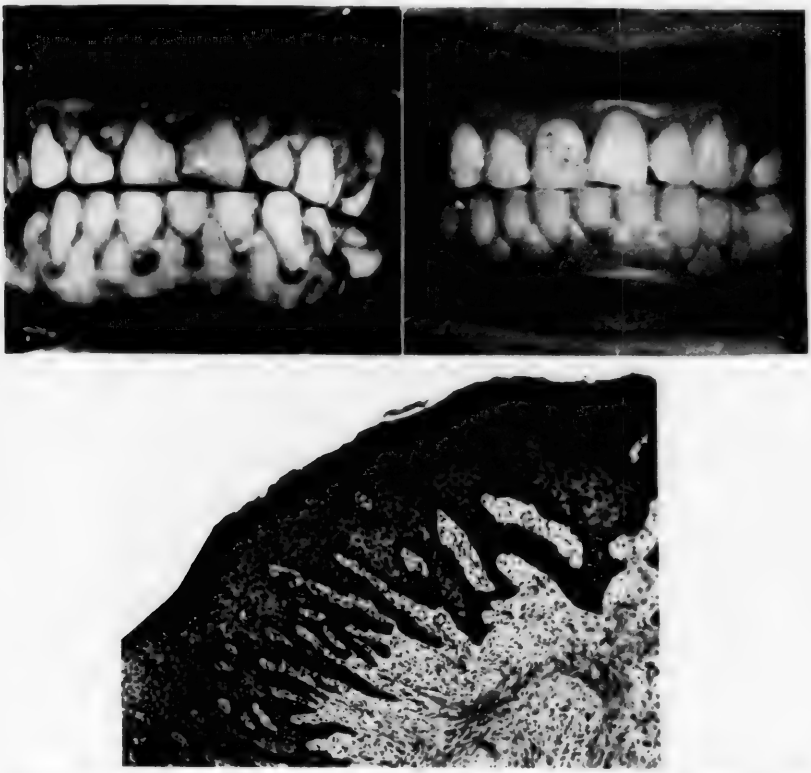

FIG. 5. Effect of conservative treatment (a) S. K. (41–4762) white female, age 30. Dilantin for 4 years, average dosage 3 grains daily. Patient noted marked hypertrophy 3 months after beginning dilantin. Surgical removal of hypertrophic tissues in 1939 was of no avail. Gingivae are hard, almost tumorous in appearance. Each interdental papilla is swollen to lobulated, cauliflower appearance. Upper median papilla is most noticeable. (b) Five months after curetting and institution of home care with emphasis on interdental stimulation. Note especially diminution in size of median papilla and increased lengths of clinical crowns. (c) Labial section of papilla between inferior left canine and second incisor. Note dipping down of rete pegs and chronic inflammatory reaction.

calculus and apoxemena were removed, occlusal trauma relieved and all marginal irritation from caries or faulty fillings eliminated. Home treatment consisted of the Stillman method of tooth brushing combined with careful interdental rubber tip massage. This regime brought about a reduction of redundant tissue to a persistent basal level not exceeded by other methods. Since further hyper-

plasia is checked and disfugurement eliminated, conservative treatment seems adequate and recommended.

SUMMARY AND CONCLUSIONS

1. A two year study of 50 selected epileptic patients receiving dilantin sodium was made.

2. Fifty-two percent of the patients showed gingival reactions; only 22 percent showed massive hyperplasia. One patient exhibited a hyperplastic lesion on the palate.

3. Gingival hyperplasia is manifested in 4 grades of severity.

4. Common local irritants to the gums act as aggravating factors to, but are not intrinsically correlated with, an already established hyperplastic tendency.

5. Increased gingival hyperplasia seems to be correlated with a longer period of dilantin administration.

6. Three methods of treatment are described for the treatment of dilantin hyperplasia, of which conservative periodontal treatment is the one recommended.

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INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

Scientific Proceedings of the New York Section[1]

January 28, 1943[2]

Compiled by Frances Krasnow, Ph.D., Editor, New York Section
Guggenheim Dental Clinic, New York, N. Y.

All papers of this meeting were to be presented at the Joint Session of the Dentistry Section, American Association for the Advancement of Science and this Section in December. They will appear in the Journal of the American College of Dentists, some of them also in the Journal of Dental Research as proceedings of the March meeting. In view of this duplication, publication of the abstracts will be omitted and continuity obtained by presentation of titles only:

1. The innervation of dentin. *Julian Ambrose,** Sydenham Hospital (see *J. D. Res.* **22:** 13, 1943).

2. Heat impact on cut vital dentin tubules by revolving instruments. *Chester J. Henschel,** *L. Lieber,** *M. Gudwin** and *J. R. Kopley,** Sydenham Hospital and Hebrew Orphan Home.

3. Further observations on dental lymph in the dentin. *William Lefkowitz*, School of Dental and Oral Surgery, Columbia University.

4. Vitamin A and estrogen interrelationship. Effect on the gingivae of monkeys of large doses of vitamin A; the ameliorating effect of the estrogenic hormone. *Daniel E. Ziskin* and *Solomon N. Rosenstein*, School of Dental and Oral Surgery, Columbia University.

5. Periodontal disease as a possible focus of infection. *Samuel Charles Miller* and *Louis R. Burman,** College of Dentistry, New York University.

6. Anthropological studies in dental caries. *Meyer Klatsky* and *J. S. Klatell,** Workmen's Circle Health Center.

7. Combination of blood sedimentation tests with short wave irradiation as an effective laboratory aid for diagnosis of active oral foci. *James L. Zemsky*, Bronx Hospital.

[1] The cost of publication of these proceedings is paid by The New York Section. Received for publication February 26, 1943.

[2] Presiding—John Oppie McCall, Guggenheim Dental Clinic.

* Guest.